University of Alberta

MERCURY BIOACCUMULATION IN FORAGE FISH COMMUNITIES INVADED BY RAINBOW SMELT (*OSMERUS MORDAX*)

by

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Abstract

This study compared total mercury concentrations ([Hg]), stable C and N isotope ratios, and growth rates among six forage fish species in 25 central Canadian lakes. Rainbow smelt (*Osmerus mordax*), an exotic species in the majority of lakes, were trophically elevated relative to most other forage species but had intermediate [Hg] and intermediate growth rates. Both among and within species, the relationship between [Hg] and trophic position was weak and negative. The strongest predictors of forage fish [Hg] were growth rate and lake conductivity; forage fish [Hg] was significantly and negatively related to both of these variables. These results indicate that although rainbow smelt may alter the trophic structure in lakes that they invade, they are unlikely to have higher [Hg] than native forage fishes and are unlikely to cause post-invasion [Hg] increases in predator fish species.

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Table of Contents

CHAPTER 1: GENERAL INTRODUCTION **1**

LITERATURE CITED 5

CHAPTER 2: TROPHIC ECOLOGY OF FORAGE FISH COMMUNITIES **9**

INTRODUCTION 9
MATERIALS AND METHODS 12
Field sampling *12*
Sample preparation and analysis *14*
Baseline correction *15*
Statistical Analysis *16*
RESULTS 18
Baseline Analyses *18*
ANCOVAs of trophic position and $\delta^{13}C$ with lake and fish size *19*
Comparisons among species and range of values *19*
Trophic position and $\delta^{13}C$ relationships with fish size *20*
Food chain length and habitat heterogeneity *21*
DISCUSSION 21
Baseline Analyses *21*
Comparisons among species *23*
Trophic position and $\delta^{13}C$ relationships with fish size *24*
Maximum trophic position and feeding diversity *25*
CONCLUSIONS 25
LITERATURE CITED 27

CHAPTER 3: FORAGE FISH GROWTH RATES AND GROWTH PATTERNS **43**

INTRODUCTION 43
MATERIALS AND METHODS 44
Field Sampling, Sample Preparation, and Sample Analysis *44*
Statistical Analyses *45*
RESULTS 47
Mass-length relationships *47*
ANCOVA of absolute growth rate with lake and fish size *47*
Comparisons among species *48*
Wet mass-age relationships *48*
Growth rate and environmental factors *49*
DISCUSSION 49
Mass-length regressions *49*
Growth rate comparisons among species and mass-age relationships *50*
Growth rate and environmental factors *50*
CONCLUSIONS 52
LITERATURE CITED 53

CHAPTER 4: MERCURY DYNAMICS IN FORAGE FISH COMMUNITIES 65

INTRODUCTION 65
MATERIALS AND METHODS 67
Field Sampling, Sample Preparation, and Sample Analysis 67
Statistical Analysis 68
RESULTS 70
ANCOVA of [Hg] with lake and fish size 70
Comparisons among species and range of values 70
Hg concentration-fish size relationships 70
Biotic Analyses 71
Abiotic Analyses 72
Combined Biotic and Abiotic Analyses 72
DISCUSSION 73
Comparisons among species and range of values 73
Hg concentration-fish size relationships 73
Abiotic, biotic, and combined analyses of Hg concentration 74
CONCLUSIONS 77
LITERATURE CITED 78

CHAPTER 5: GENERAL CONCLUSIONS 94

LITERATURE CITED 96

APPENDIX A: SUMMARY OF MERCURY CONCENTRATIONS 98

List of Tables

Table 2.1. Summary of lake characteristics 32
Table 2.2. Baseline $\delta^{15}N$ and $\delta^{13}C$ signatures 33
Table 2.3a. Summary statistics of within-species ANCOVA of trophic position (adj. $\delta^{15}N$) 34
Table 2.3b. Summary statistics of within-species ANCOVA of $\delta^{13}C$ 35
Table 2.4. Mean[1] trophic position (adj. $\delta^{15}N$) and $\delta^{13}C$ for all species captured 36
Table 2.5. Summary statistics of species-specific linear regressions between trophic position and $\log_e$ wet mass and $\delta^{13}C$ and $\log_e$ wet mass[1] 37
Table 3.1. Summary of lake characteristics 56
Table 3.2 Values of 'a' (intercept)[1] and 'b' (slope) for mass-length regressions 57
Table 3.3. Summary statistics of within-species ANCOVA of growth rate 58
Table 3.4 Mean[1] absolute growth rates for all species captured 59
Table 3.5. Summary statistics of intraspecific linear regressions between $\log_e$ wet mass and age[1] 60
Table 3.6. Summary of Pearson product-moment correlations between intraspecific growth rates and lake characteristics[1] 61
Table 4.1. Summary of lake characteristics 84
Table 4.2. Summary statistics of within-species ANCOVA of [Hg] 85
Table 4.3. Mean[1] total Hg concentrations for all species captured 86
Table 4.4. Summary statistics of intraspecific linear regressions between Hg concentration and. $\log_e$ wet mass[1] 87
Table 4.5. Summary of Pearson product-moment correlations between intraspecific $\log_e$ Hg concentrations and biotic variables 88
Table 4.6. Summary of Pearson product-moment correlations between intraspecific $\log_e$ Hg concentrations and abiotic variables 89
Table A.1 Mean total Hg concentrations by lake and species 98

List of Figures

Figure 2.1. Map of the study lakes. 38
Figure 2.2. Mean + SE baseline $\delta^{15}N$ among three classes of maximum human population density in the watershed, high (>600 people/km^2), medium (10–599.99 people/km^2), and low (<10 people/km^2). Lakes with high human population density (Lake Erie, Lake Ontario, and Lake Winnipeg (south basin)) had significantly higher baseline $\delta^{15}N$ than lakes with either medium or low maximum population density (Tukey's test, $P<0.05$). Letters indicate significant differences. 39
Figure 2.3. Linear regression of baseline $\delta^{13}C$ and $\log_e$ lake area. The relationship was significant and positive, baseline $\delta^{13}C$ increased with increasing lake area (Linear regression, $R^2=0.17$, $P=0.0265$, df=1,22). 40
Figure 2.4. Mean + SE trophic position and $\delta^{13}C$ for the six species analyzed statistically. Letters (a,b) indicate significant inter-species differences in trophic position and numbers (1,2,3) indicate significant inter-species differences in $\delta^{13}C$. At a standardized mass of 8 g, rainbow smelt and trout-perch had significantly higher trophic positions than all other species. Yellow perch had a significantly less negative $\delta^{13}C$ signature than all other species except spottail shiner, and spottail shiner had a significantly less negative $\delta^{13}C$ signature than either rainbow smelt or cisco (Tukey's test, $P<0.05$) 41
Figure 2.5a,b. Species-specific linear regressions of trophic position (a) and $\delta^{13}C$ (b) vs. $\log_e$ wet mass. Data used to obtain the relationships were corrected for among-lake differences but individual data points are not shown for clarity (N=765). Slopes were not significantly different among species for either trophic position (ANCOVA, $F=1.92$, $P=0.11$, df=5,722) or $\delta^{13}C$ (ANCOVA, $F=1.46$, $P=0.20$, df=5,765). The differences among species in trophic position and $\delta^{13}C$ are consistent across a range of body sizes. 42
Figure 3.1. Map of the study lakes. 62
Figure 3.2. Least-squared mean (standardized mass=8 g) $\log_e$ growth rates + SE. Yellow perch and cisco had significantly higher growth rates than all other species (Tukey's test, $P<0.05$) and emerald shiner had significantly lower growth rates than all other species except spottail shiner (Tukey's test, $P<0.05$). Letters indicate significant pair-wise differences. 63
Figure 3.3. Species-specific linear regressions of loge wet mass vs. age. Data used to obtain the relationships were corrected for among-lake differences but individual data points are not shown for clarity (N=778). Slopes were significantly different among species (ANCOVA, $F=19.13$, $P<0.0001$, df=5,766). It does not appear, however, that changing the size of standardization would alter the inter-species comparison of growth rate (wet mass/age); cisco and yellow perch had higher mass-at-age than all other species across the range of captured ages and spottail shiner and emerald shiner had lower mass-at-age than all other species across the range of captured ages. 64
Figure 4.1. Map of the study lakes. 90
Figure 4.2. Least-squared mean (standardized mass of 8g) total Hg concentrations + SE. Emerald shiner had significantly higher Hg concentration than all other

species, and spottail shiner had a significantly higher Hg concentration than cisco, rainbow smelt, trout-perch, and yellow perch (Tukey's test, $P<0.05$). Letters indicate significant pair-wise differences. 91
Figure 4.3. Species-specific linear regressions of total [Hg] vs. $\log_e$ wet mass. Data used to obtain the relationships were corrected for among-lake differences but individual data points are not shown for clarity (N=753). Slopes were significantly different among species (ANCOVA, F=3.78, P=0.0022, df=5,741). It does not appear, however, that changing the size of standardization would alter the inter-species comparison. 92
Figure 4.4 a,b,c. Interspecific (all species pooled) linear regressions of total Hg concentrations vs. growth rate, $\delta^{15}N$-determined trophic position, and $\delta^{13}C$, respectively. When all three variables are entered in a multiple regression to explain variation in Hg concentrations, growth rate explains the vast majority of the variation (R^2=0.22 (growth rate), 0.025 (trophic position), and 0.035 ($\delta^{13}C$). Values plotted are least-squared means calculated for each species in each lake at a standardized mass of 8g. 93

Chapter 1: General Introduction

Rainbow smelt *(Osmerus mordax)* are small, elongate silvery fish that are native to coastal regions of North America and isolated freshwater lakes of the St. Lawrence drainage (Scott and Crossman 1973). The species' freshwater range has recently expanded, however, due to a series of both accidental and intentional introductions (Evans and Loftus 1987).

Rainbow smelt were intentionally introduced into Crystal Lake, Michigan in 1912 as prey for salmonid species, and subsequently spread throughout the Great Lakes during the 1920's and 1930's (Dymond 1944, Evans and Loftus 1987). Lakes Simcoe, Nipissing, and Nipigon were invaded in 1960, 1964, and 1976, respectively (MacCrimmon et al. 1983a, Evans and Loftus 1987). Following this, three introductions in northwestern Ontario resulted in the invasion of both the English and Rainy River systems. They were reported in Lake of the Woods and Lake Winnipeg in 1990 and in lakes on the lower Nelson River between 1994 and 1996 (Campbell et al. 1991, Remnant et al. 1997). In 1999 they reached the Nelson River estuary and in summer 2002 they were reported in fish stomachs at Churchill, Manitoba (R. Remnant, North/South Consultants, Winnipeg, MB, pers. comm.). Large dams at Grand Rapids, MB, and Southern Indian Lake, MB currently limit their movement westward into the Saskatchewan and Churchill drainages, but these barriers may be easily overcome by bait-bucket or other incidental introductions.

The ecological effects of smelt invasions are difficult to predict and variable among lakes. They have the potential to interact with a wide variety of species because they are opportunistic feeders and their life history is eurythermal (Scott and Crossman 1973, Evans and Loftus 1987, Franzin et al. 1994, Hrabik and Magnuson 1999). Smelt predation has been implicated in recruitment failures of other forage (prey) fish such as lake whitefish (*Coregonus clupeaformis*) and cisco (*Coregonus artedi*) (Colby et al. 1987, Evans and Loftus 1987, Franzin et al. 1994). As well, cisco, lake whitefish, and yellow perch (*Perca flavescens*) may be negatively affected by food resource competition with rainbow smelt (Franzin et al. 1994, Hrabik et al. 1998).

Exotic smelt populations can also affect predator fish species. There is evidence that they prey on the juveniles and larvae of important sport-fish in some lakes (Colby et al. 1987, Evans and Loftus 1987). Also, they often become a preferred prey for adult walleye (*Sander vitreus*), northern pike (*Esox lucius*), and lake trout (*Salvelinus namaycush*) (Scott and Crossman 1973, Evans and Loftus 1987, Jones 1994). This can lead to increased predator growth rates (Evans and Loftus 1987, Franzin et al. 1994, Jones 1994) and, in some systems, higher concentrations of contaminants (MacCrimmon et al. 1983b, Mathers and Johansen 1985, Vander Zanden and Rasmussen 1996). Increases in predator contaminant concentrations are thought to occur because smelt feed at a higher trophic position than native forage fish. Although their diet is often dominated by zooplankton and invertebrates, they are capable of feeding on young sportfish, cisco, lake whitefish, cyprinid species, and conspecifics (MacCrimmon and Pugsley 1979, Evans and Loftus 1987, Mills et al. 1995, O'Gorman et al. 2000). Following the theory of biomagnification, this lengthening of the food chain to top predators should result in increased concentrations of biomagnifying contaminants such as mercury (Hg) (Cabana et al. 1994, Kidd et al. 1995, Vander Zanden and Rasmussen 1996).

There is mixed empirical support for the prediction that predators switching from native forage fish to smelt have higher mercury concentrations ([Hg]). A study conducted by Vander Zanden and Rasmussen (1996) on oligotrophic lakes in Ontario and Quebec found that smelt had significantly higher trophic positions (based on diet data) and [Hg] than other forage fish. Lake trout in these smelt-invaded lakes had significantly higher trophic positions (based on gut content analyses) and [Hg] than those in reference lakes (VanderZanden and Rasmussen 1996). In contrast, studies conducted in the recently-invaded Hudson Bay drainage found that although smelt were trophically elevated relative to other forage fish species (determined by stable isotopes), they had relatively low [Hg] (Swanson et al. 2003). As well, although the invasion did have a positive and significant effect on predator trophic position (determined by $\delta^{15}N$), the effect on [Hg] was not statistically significant (Johnston et al. 2003).

The results of Swanson et al. (2003) and Johnston et al. (2003) were unexpected and conflict with studies of biomagnification that predict a positive relationship between Hg concentration and trophic position (Cabana and Rasmussen 1994, Cabana et al. 1994,

Kidd et al. 1995, Vander Zanden and Rasmussen 1996). They also suggest that the effect of rainbow smelt invasion on predator [Hg] varies substantially among lakes. It is the purpose of the present study to elucidate the pattern of this variable response while investigating the decoupling of trophic position and [Hg] found by Swanson et al. (2003).

In aquatic food web studies, relationships between contaminant concentrations and trophic position are often determined and quantified by examining multiple trophic levels (e.g., from primary consumers to top predators). Swanson et al. (2003), however, examined the relationship of trophic position and [Hg] within the trophic guild of "forage fishes." It is possible that at such fine scales of trophic differentiation, factors other than trophic position determine inter-species differences in contaminant concentration. One of these possible factors is growth; in many fish species, rapid growth results in lower contaminant concentrations at a given body size or age. This can occur because exposure times to contaminants are limited in fast-growing, shorter-lived fish (de Freitas et al. 1974, Huckabee et al. 1979) and because fast-growing fish often display "growth dilution" (Meinertz 1995, Rask et al. 1996, Doyon et al. 1998).

Growth dilution occurs when fish are growing fast and efficiently. Fish with high growth efficiencies produce more flesh per unit prey intake than fish with low growth efficiencies (Wootton 1990, Jobling 1994). At a given prey and contaminant intake, these fishes with high growth efficiency and high growth rate will therefore have more flesh to dilute their absolute contaminant burden (Madenjian et al. 1994, Meinertz 1995, Rask et al. 1996, Doyon et al. 1998). Because rainbow smelt are able to feed on larger, more energy-dense prey (i.e., they can be piscivorous), they may have higher growth efficiencies and growth rates than other forage fish. If true, and depending on the relative mercury burden of the rainbow smelt diet, these high growth rates may cause rainbow smelt to have lower [Hg] than species of an equal or lower trophic position.

If growth rate is a primary determinant of forage fish [Hg], then the effects of smelt invasion on predator [Hg] will depend on relative growth rates of the forage fish species rather than relative trophic positions. These relative growth rates may be affected by many factors, including food availability, lake productivity and morphometry, temperature, social interactions, dissolved oxygen, pH, salinity, time since smelt invasion, and latitude (Copeman and McAllister 1978, Wootton 1990, Jobling 1994).

The objectives of this study were to determine: 1) if the decoupling of forage fish trophic position and [Hg] (Swanson et al. 2003) is true over a range of lake types and geographic regions; 2) the factors that best determine forage fish [Hg]; and, 3) forage fish growth rates and the relation of growth rates to environmental variables. These objectives are addressed in the following three chapters. In the first chapter, stable C and N isotopes ($\delta^{15}N$ and $\delta^{13}C$) are investigated and compared among rainbow smelt, other forage species, and baseline organisms. The second chapter is a short survey study of forage fish growth. Inter-species differences in growth rates and growth patterns are quantified and related to environmental variables. The third chapter compares [Hg] among forage fish species and relates [Hg] to $\delta^{15}N$, $\delta^{13}C$, growth rate, and environmental variables. The results of this study provide insight into food web and mercury dynamics in forage fish communities and address a paucity of data on forage fish growth.

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Chapter 2: Trophic ecology of forage fish communities

Introduction

Stable isotope analysis is a powerful tool for elucidating trophic ecology and food web structure in both aquatic and terrestrial ecosystems. Ratios of ^{15}N:^{14}N and ^{13}C:^{12}C ($\delta^{15}N$ and $\delta^{13}C$ when compared to international standards) can be used as continuous, time-integrated indicators of trophic position and carbon source, respectively (DeNiro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987). When averaged over an entire food chain, $\delta^{15}N$ signatures increase ~3.4‰ per trophic transfer because of fractionation processes during protein synthesis, assimilation, and excretion (Minagawa and Wada 1984, Ponsard and Averbuch 1999, Post 2002). Organisms at the top of the food chain therefore have higher $\delta^{15}N$ signatures than organisms at the bottom of the food chain.

Delta^{13}C signatures reflect differences in carbon source and are used in freshwater systems to differentiate between littoral and pelagic carbon sources (France 1995b, Hecky and Hesslein 1995) and between allochthonous and autochthonous carbon sources (LaZerte 1983, Rounick and Winterbourn 1986, Zah et al. 2001). The amount of $\delta^{13}C$ enrichment that occurs between predator and prey is still a topic of debate, but is generally thought to be between 0–2‰ (DeNiro and Epstein 1978, France 1995a, 1996, Post 2002). The vast majority of variation in $\delta^{13}C$ signatures is therefore attributed to differences in carbon fractionation during photosynthesis and to variation in the sources of dissolved inorganic carbon (DIC = $HCO_3^- + CO_3^{2-} + CO_{2(aq)} + H_2CO_3$).

Carbon fractionation during photosynthesis varies with the availability and source of aqueous carbon dioxide ($CO_{2(aq)}$). Benthic algae tend to have higher (less negative) $\delta^{13}C$ than phytoplankton because boundary layers that inhibit $CO_{2(aq)}$ diffusion are thicker for benthic algae than for phytoplankton. $CO_{2(aq)}$ is thus more limiting for benthic algae, and increased limitation results in decreased fractionation and increased use of bicarbonate ions (isotopically heavy compared to $CO_{2(aq)}$) (Rounick and Winterbourn 1986, France 1995b, Hecky and Hesslein 1995).

The sources of DIC to freshwater systems each have unique $\delta^{13}C$ signatures (Rounick and Winterbourn 1986, France 1995b, Hecky and Hesslein 1995). $CO_{2(aq)}$ that is respired from internal production or decomposition of terrestrial litter tends to be isotopically light (-25 to –30‰), $CO_{2(aq)}$ derived from the atmosphere is intermediate (-7‰), and dissolved carbonate and bicarbonate ions from rock weathering or buffering reactions are relatively heavy (-2 to +2‰) (Rounick and Winterbourn 1986, Hecky and Hesslein 1995). The relative importance of inorganic carbon sources varies among lakes, and positive relationships between baseline $\delta^{13}C$ and lake size have been attributed to increases in DIC $\delta^{13}C$ with lake size (Post 2002).

Stable isotope analyses improve on traditional dietary analyses by reflecting assimilated rather than ingested material, accounting for omnivory, and integrating across short-term variations in diet. A serious limitation arises, however, when $\delta^{15}N$ signatures are compared among lakes. Differences in $\delta^{15}N$ at the base of the food web (baseline $\delta^{15}N$) make comparisons among lakes difficult and have led to inflated estimates of intraspecific trophic variability (Cabana and Rasmussen 1996). Baseline $\delta^{15}N$ signatures tend to increase with human population density and fraction of residential land in the watershed. This is because the $\delta^{15}N$ of human sewage is ~10‰ higher than other sources of inorganic nitrogen (e.g., natural soils, atmospheric) (Heaton 1986, Cabana and Rasmussen 1996, Lake et al. 2001).

A number of models have been proposed to account for among-lake differences in baseline $\delta^{15}N$. The simplest model subtracts unionid mussel $\delta^{15}N$ from fish $\delta^{15}N$ (Cabana and Rasmussen 1996). Unionid mussels are an appropriate baseline indicator because they are relatively long-lived primary consumers (plankton feeders) that integrate seasonal variation in isotope signatures (Cabana and Rasmussen 1996). Post et al. (2002) developed a slightly more complex model where both unionid mussel and gastropod isotope signatures are included in a two-end-member mixing model that accounts for both seasonal and spatial (littoral vs. pelagic) variation in baseline isotope signatures. A third model for baseline correction relates primary consumer $\delta^{13}C$ to primary consumer $\delta^{15}N$. From this relationship, predictions of fish $\delta^{15}N$ are made (Vander Zanden and Rasmussen 1999). This third model must be used

with caution because it assumes a consistent and negative relationship between primary consumer $\delta^{15}N$ and $\delta^{13}C$ (Post 2002).

Fish $\delta^{15}N$ signatures vary with fish size and food chain structure as well as with baseline $\delta^{15}N$. Fish that undergo ontogenetic shifts in diet often show positive relationships between $\delta^{15}N$-determined trophic position and body size because they consume larger (and trophically elevated) prey at larger body sizes (France et al. 1998). Previous studies examining relationships between $\delta^{15}N$-determined trophic position and body size have produced mixed results. A study conducted on 13 lakes in Ontario and Québec found that there was no relationship between lake trout (*Salvelinus namaycush*) trophic position and body size (Vander Zanden et al. 2000). In contrast, trophic position-body size relationships were significant and positive in Arctic char (*Salvelinus arcticus*) in the Northwest Territories, salmonid species in Alaska, and forage fish species in northwestern Ontario (Hobson and Welch 1995, Kline et al. 1998, Swanson et al. 2003). A positive relationship between trophic position and body size was also reported for walleye (*Sander vitreus*) in Lake Champlain. In this case, however, the relationship could not be accounted for by ontogenetic shifts in diet and age was a better predictor of trophic position than length (Overman and Parrish 2001). These authors thus attributed the positive relationship between walleye trophic position and size to age-induced increases in metabolic rate and nitrogen recycling.

When applied to entire communities or ecosystems, $\delta^{15}N$ and $\delta^{13}C$ signatures can be used to quantify the length of the food chain and the degree of trophic diversity. Ecologists have been investigating factors that determine food chain length for decades. There are three main hypotheses that attempt to explain variability in food chain length, the productivity hypothesis, the productive-space hypothesis, and the ecosystem-size hypothesis. The productivity hypothesis predicts that food chain length increases with the primary productivity of the system (Pimm 1982, Kaunzinger and Morin 1998). The productive-space hypothesis predicts that food chain length increases with both lake size and primary productivity because space multiplied by productivity approximates the total energy available in the system (productivity rate*space = kg C/d) (Schoener 1989). The basis of both of these hypotheses is that

species diversity and food chain length will be limited by the amount of energy available in the system.

The basis of the ecosystem-size hypothesis is that habitat heterogeneity and complexity increase with ecosystem size, and this leads to increases in species diversity and food chain length (maximum trophic position) (Holt 1993, Post et al. 2000). A recent study conducted on lakes in New York found that lake volume explained 80% of the variation in food chain length; productivity was not significant (Post et al. 2000). Another study found that both lake area and productive-space were positively correlated with food chain length, but the productive-space relationship was very similar to the lake area relationship. This again suggests that ecosystem size is the primary determinant of food chain length (Vander Zanden et al. 1999).

The objectives of this study were to determine: 1) the trophic ecology of a suite of forage fish species; 2) trophic position-fish size relationships; 3) the relationship between baseline $\delta^{15}N$ and human population density; 4) the relationship between baseline $\delta^{13}C$ and lake area; and, 5) the effect of lake size on maximum trophic position and feeding diversity in forage fish communities in central Canadian lakes.

Materials and Methods

Field sampling

Twenty-five lakes with established rainbow smelt populations were sampled during the open-water season of 2002 (Table 2.1). Three of these lakes had native smelt populations (Lac Heney, Muskrat Lake, Lake Champlain) while the remainder had naturalized populations. The lakes covered a broad geographic area, from Lake Champlain, Vermont (44°30', 73°12'), to Stephens Lake, Manitoba (56°23', 96°55') (Figure 2.1). University of Alberta personnel sampled 16 of the lakes. The Department of Fisheries and Oceans, North/South Consultants, Vermont Fish and Wildlife, and the Ontario Ministry of Natural Resources sampled the remainder.

In each lake, inshore and offshore locations close to the main basin were sampled for forage fish species. Most fish were collected with small-mesh (½''–1 ½'') monofilament gill nets set at depths ranging from 2 m to 30 m. An otter trawl

was used to capture offshore species in the Great Lakes and Lake Champlain. Captured species included rainbow smelt (*Osmerus mordax*), cisco (*Coregonus artedi*), yellow perch (*Perca flav escens*), trout-perch (*Percopsis omiscomaycus*), spottail shiner (*Notropis hudsonius*), emerald shiner (*Notropis atherinoides*), common shiner (*Luxilis cornutus*), golden shiner (*Notemigonus crysoleucas*), pumpkinseed (*Lepomis gibbosus*), rock bass (*Ambloplites rupestris*), round goby (*Neogobius melanostomus*), slimy sculpin (*Cottus cognatus*), and alewife (*Alosa pseudoharengus*). Each lake was represented by samples of rainbow smelt and up to four other forage species. Sample sizes were approximately 10 individuals / species / lake. When more than 10 individuals were captured, fish that represented a large size range were selected for analysis. The total sample size (all forage fish in all lakes) was 977.

Unionid clams (*Pyganodon grandis* and *Lampsilis radiata*) or zebra mussels (*Dreissena polymorpha*) were collected from each lake to establish lake-specific baseline signatures for $\delta^{15}N$ and $\delta^{13}C$. Both of these primary consumers are relatively long-lived and integrate much of the seasonal variation that is present in primary producer isotope signatures. Also, zebra mussel and clam isotope signatures can be directly compared as long as the largest, oldest clams are avoided (Post 2002). Unionid clams were collected with a dip net or by snorkelling and zebra mussels (*Dreissena polymorpha*) were collected with an otter trawl. The target sample size was 10 clams / lake or 30 zebra mussels / lake.

Clams could not be captured in Lake Winnipeg, Split Lake, or Stephens Lake. Clam isotope data for Lake Winnipeg was obtained from the Department of Fisheries and Oceans (K.Kidd, pers. comm.). In Split Lake, Stephens Lake, and Gull Lake (for comparison), burrowing mayflies (*Hexagenia* sp.) were collected with an Eckman grab. The target sample size was 30 individuals / lake.

In lakes that were sampled by the University of Alberta, conductivity and pH were measured with a Hydrolab Surveyor 4 meter. Lakes that were not sampled by the University of Alberta were either excluded from analyses that included these variables (Great Lakes) or conductivity and pH data were obtained from collaborative sampling crews. Topographic maps, bathymetric maps, and an Ontario Ministry of

Natural Resources database were used to determine latitude, longitude, lake area, and maximum depth of each lake. Using population density maps, each lake was also assigned a class that represented maximum population density in the watershed. The maximum population densities of the "high", "medium", and "low" categories were >600 people/km^2, 10 to 599.99 people/km^2, and <10 people/km^2, respectively. Lake characteristic data are summarized in Table 2.1.

Sample preparation and analysis

Following capture, all forage fish, clam, zebra mussel, and mayfly samples were held on ice until they could be frozen whole and transported to the laboratory. They were then thawed at room temperature and blotted to remove excess moisture. Fish were measured for fork length, total length, and wet mass while clams, zebra mussels, and mayflies were measured for length (either shell or total) and wet mass. Fish dorsal muscle tissue was excised for Hg (see Chapter 4) and stable isotope analysis and clam and zebra mussel foot tissue was excised for stable isotope analysis only. Whole mayflies were used for stable isotope analysis. All samples were freeze-dried for 48 hours at 134 millitorr and -50°C before being ground to a fine powder using a mortar and pestle. Subsamples of this dried powder were then analyzed for total mercury concentrations (fish, Chapter 4) and stable carbon and nitrogen isotope ratios (fish, clams, zebra mussels, and mayflies). For zebra mussels, composite samples of up to five individuals were homogenized to obtain an adequate mass for analysis.

Analyses of stable C and N isotopes were performed on a Finnigan Mat Delta Plus continuous-flow isotope-ratio mass spectrometer connected to a Thermoquest NC2500 elemental analyzer (EA-CFIRMS) at the University of New Brunswick. Carbon and nitrogen content were analyzed simultaneously with the elemental analyzer and converted to mole ratios of carbon to nitrogen (C/N). Stable isotope ratios were expressed as parts per mil (‰) delta values ($\delta^{15}N$ and $\delta^{13}C$) from an international standard. The standard for carbon is Pee-Dee Belomnite and the standard for nitrogen is atmospheric nitrogen gas (Peterson and Fry 1987). The delta values were calculated using the following formula:

1. $$\delta^{15}N \text{ or } \delta^{13}C = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000$$

where $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$. A series of five internal standards were analyzed every 24 samples for a total of 76 samples and 20 standards per run. Internal precision for standards was ± 0.2 ‰. The mean difference ± SD between duplicate sub-samples was 0.07 ± 0.4‰ (n=80) for $\delta^{15}N$ and 0.02 ± 0.2‰ (n=80) for $\delta^{13}C$.

Baseline correction

Delta^{15}N was used as an index of trophic position in this study. As discussed above, however, among-lake differences in $\delta^{15}N$ at the base of the food web necessitate a baseline correction (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post 2002). This is usually accomplished by standardizing fish $\delta^{15}N$ to the $\delta^{15}N$ of standard primary consumers such as clams, zebra mussels, and/or gastropods. I could not use the baseline correction model proposed by Post et al. (2002) because adequate gastropod samples could not be collected from many lakes. I chose not to use the model proposed by Vander Zanden and Rasmussen (1999) because there was not a consistent and negative relationship between primary consumer $\delta^{15}N$ and $\delta^{13}C$ in the lakes that I studied (a necessary assumption for this model). I therefore used a modified version of the model proposed by Cabana and Rasmussen (1996). Lake-specific clam (or zebra mussel) $\delta^{15}N$ was subtracted from fish $\delta^{15}N$. I did not divide by the conventional 3.4‰, however, as this estimate of fractionation is only valid when applied to entire food webs or multiple trophic interactions (Post 2002). Adjusted $\delta^{15}N$ values (trophic position) were calculated for each lake as follows:

2. $$\text{adjusted } \delta^{15}N \text{ (trophic position)} = \delta^{15}N_{fish} - \delta^{15}N_{baseline}$$

where $\delta^{15}N_{baseline}$ was either a lake-specific least-squared mean (LSMean) of clam $\delta^{15}N$ (standardized clam mass = 37g) or a lake-specific arithmetic mean of zebra mussel $\delta^{15}N$. Least-squared means were necessary for the calculation of clam $\delta^{15}N$ because clam $\delta^{15}N$ was significantly related to clam mass.

Comparisons of burrowing mayfly (*Hexagenia* sp.) $\delta^{15}N$ and $\delta^{13}C$ revealed that isotope signatures in Split Lake and Stephens Lake did not differ significantly from those in Gull Lake (Tukey's test, $P>0.05$). This was expected because Split Lake and Stephens Lake are immediately upstream and downstream of Gull Lake, respectively (Figure 2.1). The clam isotopic baseline obtained for Gull Lake was therefore applied to Split Lake and Stephens Lake.

Statistical Analysis

There were two criteria for selecting species to be included in the statistical analysis. First, the species had to be caught in 5 or more lakes. Second, the set of lakes where that species was captured had to reflect a random sub-sample of all lakes sampled (e.g., not all southern lakes, not all small lakes). Based on these criteria, rainbow smelt, yellow perch, spottail shiner, trout-perch, emerald shiner, and cisco were included in the statistical analysis. To enable general comparisons, however, means were calculated and presented for all species, including those excluded from subsequent statistical analysis.

Statistical analyses on the 6 selected species included linear regression, analysis of covariance (ANCOVA), and analysis of variance (ANOVA) (GLM procedure, REG procedure, SAS Institute Inc., 1990). After each statistical analysis, residual plots were examined for homogeneity of variance and both Shapiro-Wilk and Kolmogorov-Smirnoff statistics (tests of normality) were calculated and found to have P-values >0.05. To achieve homogeneity of variance, wet mass was $\log_e$ (ln) transformed prior to analysis.

Analyses of covariance were used to determine if there were species-specific differences in isotope ratios among lakes, and if isotope ratios were related to body size. They were also used to standardize all clam and fish isotope ratios to a common body size (see similar procedures in Chapters 3 and 4). This allowed for comparisons among populations or species where different size distributions of organisms were captured. Zebra mussel isotope ratios could not be standardized to a common body size because the samples were homogenates of many individuals.

For both fish and clam ANCOVA models, lake was the categorical variable and $\log_e$ wet mass was the continuous variable. Wet mass was chosen as the size

covariate because mass data are less sensitive to differences in inter-species morphometry than length data. Fish least-squared means of trophic position (adjusted $\delta^{15}N$) and $\delta^{13}C$ were calculated for each species in each lake at a mass of 8g. Clam least-squared means of trophic position and $\delta^{13}C$ were calculated in each lake at a mass of 37g. These sizes were chosen because they were within the size range that was captured in most lakes and they were also close to the overall median size captured. As well, 8g is a reasonable prey size for predators such as walleye (Knight and Vondracek 1993).

Once trophic position and $\delta^{13}C$ least-squared means were calculated for fish, they were compared among species with one-way ANOVA. Lake was included in the model as a block effect and a post-hoc Tukey's test was performed to identify significant pair-wise differences. To ensure that inter-species differences in $\delta^{13}C$ did not arise from differences in lipid content, least-squared mean C/N ratios (a surrogate for lipid content (Tieszen et al. 1983)) were also compared among species with an ANOVA and C/N ratios were related to $\delta^{13}C$ with a linear regression.

Linear regressions were used to further investigate the effect of body size on trophic position and $\delta^{13}C$ and to assess if changing the body size of comparison (8g) (within the range of captured body sizes common to all species) would affect the results of inter-species comparisons. To do this, among-lake differences in trophic position and $\delta^{13}C$ were determined for each species with an ANOVA. Species-specific residuals from this model were then regressed against $\log_e$ wet mass and slopes and intercepts of the relationships were compared among species.

To determine the relationship of the isotopic baseline to lake characteristics, baseline $\delta^{15}N$ was compared among human population density classes (low, medium, high) with one-way ANOVA. A post-hoc Tukey's test was performed to determine significant pair-wise differences. Following this, baseline $\delta^{13}C$ was related to lake area with a linear regression.

The final objective of this study was to determine if there were relationships between lake area (surrogate for ecosystem size) and either maximum trophic position or feeding diversity in the forage fish community. To test this, maximum trophic positions (adjusted $\delta^{15}N$ signature) were calculated in each lake both within and

among species. Feeding diversity was quantified as the coefficient of variation of $\delta^{13}C$ and was calculated both within and among species in each lake. Maximum trophic position and feeding diversity were then regressed against $\log_e$ lake area for both intraspecific and interspecific analyses.

Results

Baseline Analyses

Two species of unionid clam were captured in this study. To ensure that this did not affect the calculation of the stable isotope baseline, both species of clam were collected from one lake and their $\delta^{15}N$ and $\delta^{13}C$ signatures were compared. Delta^{15}N and $\delta^{13}C$ did not differ significantly between species (ANOVA, $F<4.336$, $P>0.071$, df=1,8).

When zebra mussel and clam data were pooled and compared among lakes, baseline $\delta^{15}N$ ranged from 1.27‰ in Lake Nipigon to 11.80‰ in Lake Erie (east basin) and the differences among lakes were significant (ANOVA, $F=306.09$, $P<0.0001$, df=20,154) (Table 2.2). A post-hoc Tukey's test revealed that baseline $\delta^{15}N$ signatures clustered into approximately 5 groups (Table 2.2). Lake Erie (east basin), Lake Ontario, and Lake Winnipeg (south basin) had significantly higher baseline $\delta^{15}N$ than all other lakes. Of the remaining lakes, Lake Erie (west basin), Lake Simcoe, and Muskrat Lake had significantly higher baseline $\delta^{15}N$ and Lake Nipigon had significantly lower baseline $\delta^{15}N$ (Tukey's test, $P<0.05$, Table 2.2).

Baseline $\delta^{13}C$ ranged from –22.30‰ in Lake Erie (west basin) to –30.19‰ in Birch Lake (Table 2.2). There were significant differences among lakes (ANOVA, $F=170.61$, $P<0.0001$, df=21,161). Lake Erie (west basin) and Rainy Lake had the least negative baseline $\delta^{13}C$ and were significantly different from most other lakes. Birch Lake, Ahmic Lake, and Minnitaki Lake had the most negative baseline $\delta^{13}C$ and were significantly different from most other lakes (Tukey's test, $P<0.05$) (Table 2.2). The remaining lakes had intermediate baseline $\delta^{13}C$ and were not significantly different from each other (Tukey's test, $P>0.05$) (Table 2.2).

Baseline $\delta^{15}N$ varied significantly with respect to human population density in the watershed (ANOVA, $F=27.69$, $P<0.0001$, df=2,21). Lakes situated in

watersheds with high maximum population density (≥600 people/km^2) had significantly higher baseline δ^{15}N than lakes with medium (10 to 599.99 people/km^2) or low (≤10 people/km^2) population density in the watershed (Tukey's test, $P<0.05$) (Figure 2.2). There was no significant difference, however, between medium and low population density classes (Tukey's test, $P>0.05$) (Figure 2.2). When mean baseline δ^{13}C was regressed against $\log_e$ lake area, the relationship was significant and positive (Linear regression, $R^2_{adj}=0.17$, $P=0.0265$, df=1,22). Larger lakes had significantly less negative baseline δ^{13}C than smaller lakes (Figure 2.3).

ANCOVAs of trophic position and δ^{13}C with lake and fish size

Analyses of covariance performed on trophic position (adjusted δ^{15}N) and δ^{13}C revealed that there were significant differences among lakes in intraspecific trophic position and δ^{13}C (statistics in Table 2.3a, 2.3b). In four of six species there were significant positive relationships between trophic position and $\log_e$ wet mass but only one species showed a significant relationship between δ^{13}C and $\log_e$ wet mass. The interaction terms ($\log_e$ wet mass*lake) were significant for two intraspecific trophic position models and three intraspecific δ^{13}C models (Table 2.3a, 2.3b). For these species, the relationship between trophic position or δ^{13}C and $\log_e$ wet mass varied among lakes and comparisons among populations should only be made at a standardized fish mass.

Comparisons among species and range of values

When all 977 fish are considered, trophic position (adjusted δ^{15}N) ranged from 0.432‰ (yellow perch in Lake Erie) to 9.52‰ (rainbow smelt in Lac Heney). All large rainbow smelt caught in Lac Heney had trophic positions greater than 8.90‰. Delta^{13}C ranged from –32.22‰ (trout-perch in Birch Lake) to –16.17‰ (spottail shiner in Lake Ontario). Most fish caught in the Great Lakes or Lake Simcoe had δ^{13}C signatures >-22‰ and all fish with δ^{13}C signatures >-20‰ were caught in the Great Lakes or Lake Simcoe.

Comparing all species captured, rainbow smelt, trout-perch, and slimy sculpin had similar and high mean trophic positions while round goby had the lowest mean trophic position (Table 2.4). Round goby also had the least negative δ^{13}C signature

while cisco, bluegill, and golden shiner had the most negative $\delta^{13}C$ signatures (Table 2.4).

At a standardized mass of 8g, there were significant differences in trophic position and $\delta^{13}C$ among species (ANOVA, $F>9.23$, $P<0.0001$, df=5,52 and 5,56). Species differences, along with a lake block variable, explained 73% ($R^2=0.73$) of the variation in trophic position and 79% ($R^2=0.79$) of the variation in $\delta^{13}C$. A post-hoc Tukey's test for trophic position revealed that rainbow smelt and trout-perch were significantly trophically elevated relative to all other species (Tukey's test, $P<0.05$) but were not significantly different from each other (Tukey's test, $P>0.05$) (Figure 2.4). Significant pair-wise differences in $\delta^{13}C$ were found between yellow perch and all other species except spottail shiner and between spottail shiner and both rainbow smelt and cisco (Tukey's test, $P<0.05$). Both yellow perch and spottail shiner had significantly higher (less negative) $\delta^{13}C$ than other species (Figure 2.4). There were no significant differences in least-squared mean C/N ratios among species (ANOVA, $F=0.46$, $P=0.81$, df=5,80) and no significant relationship between least-squared mean $\delta^{13}C$ and least-squared mean C/N (Linear regression, $R^2=0.03$, $P=0.42$, df=1,84).

Trophic position and $\delta^{13}C$ relationships with fish size

To determine if changing the body size of comparison (8g) would alter the differences in trophic position and $\delta^{13}C$ among species, regressions of trophic position and $\delta^{13}C$ vs. $\log_e$ wet mass were computed and compared among species (the lake effect was first removed). Rainbow smelt and trout-perch were trophically elevated across the range of captured body sizes common to all species (Figure 2.5a). The only pair-wise comparison that would change with body size was between yellow perch and spottail shiner; spottail shiner had a slightly higher trophic position than yellow perch above 8g ($\log_e$ wet mass = 2) but a slightly lower trophic position than yellow perch below 8g.

The inter-species comparisons of $\delta^{13}C$ were also consistent across the range of captured body sizes common to all species. The only two species with intersecting relationships were rainbow smelt and emerald shiner. At the lower end of the captured size range, emerald shiner had an equal or slightly more negative $\delta^{13}C$

signature than rainbow smelt. Their relative positions reversed, however, at the upper end of the captured size range (Figure 2.5b).

In agreement with the fact that inter-species differences were consistent across the range of captured body sizes, slopes of trophic position-$\log_e$ wet mass and $\delta^{13}C$-$\log_e$ wet mass relationships were not significantly different among species (ANCOVA, $F<1.82$, $P>0.11$, df=5,722) (Figure 2.5a,b). Relationships between trophic position and mass were positive for all species and significant for all species except emerald shiner (Table 2.5). Delta^{13}C was positively related to $\log_e$ wet mass in all species except cisco, and the relationships were significant in emerald shiner, rainbow smelt, and yellow perch. Regression statistics for both trophic position and $\delta^{13}C$ are summarized in Table 2.6.

Food chain length and habitat heterogeneity

Linear regressions of maximum trophic position and $\log_e$ lake area revealed that maximum trophic position did not increase with lake area; this was true for both interspecific (Linear regression, $R^2=0.06$, $P=0.27$, df=1,21) and intraspecific (Linear regression, $R^2<0.42$, $P>0.09$) analyses. With all species pooled in the interspecific analysis of feeding diversity, there was a positive and marginally significant ($P<0.1$) relationship with lake area (Linear regression, $R^2=0.14$, $P=0.089$, df=1,21). Within species, however, relationships between feeding diversity and lake area were not always positive and were not significantly related to lake area (Linear regression, $R^2<0.4$, $P>0.1$).

Discussion

Baseline Analyses

Baseline $\delta^{15}N$ was highest in lakes that had high human population density in the watershed. Lake Winnipeg (south basin), Lake Erie (east basin), and Lake Ontario all had high human population density and significantly elevated baseline $\delta^{15}N$ (~11‰). Previous authors have found that baseline $\delta^{15}N$ signatures of ~11‰ are typical of lakes with >1000 people/km^2 in the watershed and attributed high baseline $\delta^{15}N$ in these systems to high human sewage inputs (Cabana and Rasmussen 1996). These high baseline signatures could also be related to agriculture, however. Studies

of soil, surface water, and groundwater have found that nitrate $\delta^{15}N$ signatures are highest in areas that receive animal waste (10 to 22‰) (e.g., feedlots, lagoons, septic systems, barnyards) (Kreitler 1975, Heaton 1986, Komor and Anderson 1993). To a lesser degree, and depending on soil type and climatic conditions, areas fertilized by inorganic nitrogen fertilizers may also have elevated nitrate $\delta^{15}N$ (Kreitler 1979). Whether the source is human sewage, feedlots, or crop agriculture, however, it appears that Lake Erie (east basin), Lake Ontario, and Lake Winnipeg (south basin) are subject to substantial inputs of anthropogenically-derived nitrogen.

The high baseline $\delta^{15}N$ result is especially interesting for Lake Winnipeg because there has been recent concern over eutrophication trends in this lake. Studies have shown that anthropogenic nitrogen inputs and phytoplankton biomass have increased dramatically in the last 30-50 years with concurrent changes in phytoplankton species composition (Kling et al. 2002, Mayer et al. 2002). The current eutrophication trend in Lake Winnipeg has been compared to the trend seen in Lake Erie in the 1970's (Sellers 2002). The baseline $\delta^{15}N$ results from the current study provide further evidence that Lake Winnipeg (south) is receiving large amounts of anthropogenic nitrogen, and that the magnitude of the problem is comparable to that in the Great Lakes.

There was a significant and positive relationship between baseline $\delta^{13}C$ and lake area. This is consistent with a previous study conducted by Post (2002). As discussed in the introduction, $\delta^{13}C$ signatures are less negative when $CO_{2(aq)}$ concentrations are limiting or when the $\delta^{13}C$ signature of the DIC pool is less negative. The $\delta^{13}C$ signature of the DIC pool will be less negative when the dominant sources of inorganic carbon are atmospheric CO_2 or rock weathering and more negative when the carbon is re-mineralized or respired from terrestrial litter or internal production (LaZerte 1983, Rounick and Winterbourn 1986, Hecky and Hesslein 1995). Post (2002) suggested that the dominant sources of DIC in smaller lakes were respiration and/or re-mineralization of organic matter and that the dominant sources of DIC in larger lakes were atmospheric CO_2 and carbonate ions derived from weathering. My results are consistent with this.

Comparisons among species

In agreement with previous studies, I found that rainbow smelt and trout-perch were significantly trophically elevated relative to cisco, spottail shiner, emerald shiner, and yellow perch (Overman and Parrish 2001, Swanson et al. 2003). As discussed by Swanson et al. (2003), the trophic elevation of rainbow smelt is not surprising because they are known to be cannibalistic and piscivorous at large sizes (MacCrimmon and Pugsley 1979, Evans and Loftus 1987). It is not currently known, however, why trout-perch are trophically elevated. Diet data are sparse, but trout-perch are believed to feed on zooplankton, chironomid larvae, ephemeropteran larvae, amphipods, and some small fish (Scott and Crossman 1973, Staggs and Otis 1996). Swanson et al. (2003) suggested that either fish contributed more to the diet of trout-perch than was previously thought, or that their standardization of trophic position to a body size of 10g resulted in an extrapolation error. The results of this study suggest that there was no extrapolation error. To fully explain their elevated trophic position, detailed diet analyses of trout-perch and stable isotope analyses of their prey items would be required.

Inter-species comparisons of $\delta^{13}C$ showed that cisco, trout-perch, emerald shiner, and rainbow smelt had more negative $\delta^{13}C$ signatures than either yellow perch or spottail shiner. These results were expected and indicate that yellow perch and spottail shiner feed primarily on benthic food sources while cisco, trout-perch, emerald shiner, and rainbow smelt feed primarily on pelagic food sources (France 1995b). Some authors have reported that because lipid is depleted in ^{13}C (DeNiro and Epstein 1977), inter-species differences in $\delta^{13}C$ may be caused by differences in proximate composition (Wada et al. 1987). France (1996) has shown, however, that this effect is more important in marine systems than in freshwater systems. My results are consistent with this; there were no differences in C/N ratios among species. Also, there was no relationship between $\delta^{13}C$ and C/N ratios. If inter-species differences in $\delta^{13}C$ were driven by differences in lipid there would be a negative relationship between $\delta^{13}C$ and C/N ratios.

When all species are considered (not just the 6 analyzed statistically), round goby had the lowest trophic position and the least negative $\delta^{13}C$. Round goby are

invasive to the Great Lakes area and are known to feed on benthic invertebrates, zooplankton, zebra mussels (*Dreissena polymorpha*), and other molluscs (Ray and Corkum 1997, French and Jude 2001, Diggins et al. 2002). Zebra mussels and round goby are both native to the Black and Caspian Sea basins and one study showed that more than 50% of round goby diet can be composed of zebra mussels (Ray and Corkum 1997). This may explain their low trophic position. If the round goby caught in this study were feeding directly on zebra mussels (the baseline organism in this study), their trophic position would be equal to the nitrogen fractionation between zebra mussels and themselves. Although the average fractionation of nitrogen over many trophic levels is 3.4‰ per level, it has been shown to range from ~0 to 6‰ over single trophic transfers (Adams and Sterner 2000). The fractionation of nitrogen between zebra mussels and round goby could therefore be very low; this would explain the low trophic position of round goby.

Trophic position and $\delta^{13}C$ relationships with fish size

Regressions of trophic position and $\delta^{13}C$ vs. fish size revealed that changing the size of comparison (8g) within the range of captured body sizes would not alter the differences observed among species. Across the range of captured body sizes, rainbow smelt and trout-perch were trophically elevated relative to the other four species and yellow perch and spottail shiner had the least negative $\delta^{13}C$.

All of the species showed positive relationships between trophic position and body size. There can be two reasons for this, ontogenetic shifts in feeding (e.g., piscivory, cannibalism) (Hobson and Welch 1995, Kline et al. 1998), or increased recycling of nitrogen with age (Overman and Parrish 2001). Some species, such as yellow perch and rainbow smelt, had slopes of similar magnitude for trophic position and $\delta^{13}C$. This may be an indication of ontogenetic shifts in feeding. Other species, such as spottail shiner, had much steeper slopes for trophic position than for $\delta^{13}C$. This could mean that large and small spottail shiners feed on organisms that differ in trophic position but not in $\delta^{13}C$ (i.e. their trophic ecology shifts vertically but not horizontally), or that there is increased nitrogen recycling with increased size and age.

Without detailed diet-at-age data, it is impossible to tell which process is responsible or dominant for any of the species.

Maximum trophic position and feeding diversity

Contrary to previous studies, I found no significant relationship between maximum trophic position and lake area. Post et al. (2000) found a significant and positive food chain length-(maximum trophic position) ecosystem size relationship that was caused by additional top predator species in larger lakes and increases in species-specific trophic position with increasing lake size. It was suggested that species-specific increases in predator trophic position were due to reduced omnivory at all levels of the food chain and increased diversity of forage species in larger lakes. In this study, interspecific analyses were performed on the same six species. Therefore, the diversification of forage fish species and addition of trophically elevated forage species in larger lakes would not be detected. If I had found a positive relationship between interspecific maximum trophic position and lake area, it would have been due to reduced omnivory and/or increased piscivory of rainbow smelt and trout-perch. Intraspecific analyses revealed, however, that maximum trophic position was not related to lake area in any of the species. It therefore appears that in the lakes included in this study, omnivorous feeding habits in forage fish do not decrease with increasing lake size.

Post et al. (2000) hypothesized that omnivory would decrease (and food chain length therefore increase) in larger lakes because they support a greater diversity of habitats and refugia. Relationships between feeding diversity and lake size were not significant in this study, but the interspecific relationship was positive and close to significant (P=0.09). If a greater number of species had been analyzed, and if the species assemblage analyzed was not the same for all lakes, I might have been more likely to find significant feeding diversity-lake size and maximum trophic position-lake size relationships. Alternatively, these previously documented relationships may be based entirely on the trophic ecology and species assemblage of predator fish.

Conclusions

The results of this study indicate that rainbow smelt and trout-perch are significantly trophically elevated relative to other forage fish species and that this

trophic elevation occurs over a wide range of body sizes. Rainbow smelt thus have the potential to lengthen the food chain to top predators. The degree of food chain lengthening, however, will depend on the species and size composition of pre- vs. post-smelt invasion diet. For instance, a switch in feeding from trout-perch to rainbow smelt would not result in a lengthened food chain to top predators.

Analyses of baseline isotope signatures indicate that baseline $\delta^{15}N$ and $\delta^{13}C$ are dependent on human population density and lake size, respectively. These analyses also indicate that Lake Winnipeg (south basin), Lake Erie (west basin), and Lake Ontario are subject to considerable anthropogenic nitrogen inputs.

Maximum trophic position and feeding diversity were not related to lake size in this study, but this could be due to the limited species assemblage analyzed and the fact that the number of species was held constant for all lakes. Alternatively, predator species may have to be included to detect these relationships.

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Table 2.1. Summary of lake characteristics

Lake	Lat.	Long.	Conductivity (µS/cm)	Max. Depth (m)	Area (km^2)	pH	Invasion Year[1]	Maximum Population Density (people/km^2)
Ahmic	45°37'	79°42'	36	27	16	7.9	1981	Medium
Birch	46°18'	81°58'	26	27	11	7.5	1980	Low
Champlain	44°30'	73°12'	220	129	1140	7.8	Native	High
Gull	56°19'	95°22'	98	.	70	8.0	1996	Low
Heney	45°57'	75°57'	131	33	12	8.2	Native	Low
Manitou	45°47'	82°00'	254	49	105	8.2	1950	Low
Minnitaki	49°58'	92°00'	57	49	181	8.2	1991	Low
Muskoka	45°00'	79°25'	46	67	90	7.9	1950	Medium
Muskrat	45°40'	76°55'	291	64	12	8.6	Native	Medium
Nipigon	49°50'	88°58'	134	137	4481	7.6	1976	Low
Nipissing	46°19'	79°28'	69	52	873	7.8	1964	Low
Rainy	48°36'	93°24'	55	49	881	8.4	1990	Low
Red	51°03'	93°57'	57	43	177	8.1	1980	Low
Round	45°38'	77°30'	58	55	31	7.9	1989	Medium
Simcoe	44°37'	79°25'	360	42	725	6.9	1960	Medium
Trout	46°20'	79°27'	93	68	16	7.8	1964	Low
Winnipeg (N)	53°11'	99°16'	371	36	21925	8.6	1990	Low
Winnipeg (S)	50°48'	96°59'	212	11	2475	.	1990	High
Lake of the Woods	49°43'	94°49'	.	66	4350	.	1990	Low
Erie (E)	42°47'	80°12'	.	64	7375	.	1935	High
Erie (W)	41°50'	82°45'	.	10	3975	.	1935	High
Huron	43°45'	81°43'	.	228	59570	.	1931	Medium
Ontario	43°15'	79°04'	.	224	19009	.	1929	High
Split	56°08'	96°15'	191	.	280	8.0	1996	Low
Stephens	56°23'	96°55'	201	.	299	8.0	1996	Low

[1]Invasion year is the first report of rainbow smelt in a lake.

Table 2.2. Baseline $\delta^{15}N$ and $\delta^{13}C$ signatures

Lake	Baseline organism[1]	Baseline $\delta^{15}N$ (‰)	Significant differences[3]	Baseline $\delta^{13}C$ (‰)	Significant differences
Ahmic Lake	Clam	5.01	DE	-29.86	A
Birch Lake	Clam	4.84	DE	-30.19	A
Lake Erie (east)	Zebra mussel	11.8	A	-24.33	EF
Lake Erie (west)	Zebra mussel	8.22	C	-22.30	F
Gull Lake	Clam	5.38	DE	-25.53	E
Lac Heney	Clam	4.53	DE	-25.25	EF
Lake Huron	Zebra mussel	3.60	F	-25.56	E
Lake Manitou	Clam	2.51	G	-26.45	DE
Lake Minnitaki	Clam	3.70	EF	-30.29	A
Lake Muskoka	Clam	5.13	DE	-26.47	CDE
Muskrat Lake	Clam	7.94	C	-29.27	AB
Lake Nipigon	Clam	1.27	H	-27.47	DE
Lake Nipissing	Clam	4.03	EF	-25.53	DE
Lake Ontario	Zebra mussel	11.3	AB	-26.20	DE
Rainy Lake	Clam	4.04	F	-24.90	F
Red Lake	Clam	3.21	FG	-29.59	AB
Round Lake	Clam	3.23	F	-28.48	ABCD
Lake Simcoe	Zebra mussel	8.25	C	-27.42	ABCDE
Split Lake	Clam[2]	5.38	DE	-25.53	E
Stephens Lake	Clam[2]	5.38	DE	-25.53	E
Trout Lake	Clam	3.55	F	-26.68	BCDE
Lake Winnipeg (north)	Clam	3.18	FG	-28.39	ABCDE
Lake Winnipeg (south)	Clam	10.6	B	-29.43	ABCDE
Lake of the Woods	Clam	5.22	D	-28.66	ABCDE

[1]Clam isotope signatures are least-squared means calculated for each lake (standardized mass=37g). Zebra mussel isotope signatures are arithmetic means calculated for each lake.
[2]Split Lake and Stephens Lake were assigned the same isotope signatures as Gull Lake.
[3]Significant differences among lakes were determined with a Tukey's test and considered significant if $P \leq 0.05$. Letters indicate significant differences.

Table 2.3a. Summary statistics of within-species ANCOVA of trophic position (adj. $\delta^{15}N$)

Species	Variable	F-statistic[2]	P-value	df
Cisco	lake	114.4	0.0001*	4,33
	wet mass[1]	30.68	0.0001*	1,33
	lake*wet mass	0.93	0.4601	4,33
Emerald shiner	lake	38.27	0.0001*	5,38
	wet mass	0.19	0.6686	1,38
	lake*wet mass	0.83	0.1193	5,38
Rainbow smelt	lake	3.46	0.0001*	21,165
	wet mass	8.90	0.0033*	1,165
	lake*wet mass	2.25	0.0024*	21,165
Spottail shiner	lake	53.71	0.0001*	11,83
	wet mass	26.53	0.0001*	1,83
	lake*wet mass	1.60	0.1145	11,83
Trout-perch	lake	3.10	0.0003*	15,110
	wet mass	1.26	0.2638	1,110
	lake*wet mass	2.53	0.0024*	15,110
Yellow perch	lake	42.56	0.0001*	19,143
	wet mass	51.45	0.0001*	1,143
	lake*wet mass	1.58	0.0676	19,143

[1]Wet mass was $\log_e$ transformed prior to analysis.
[2]F-statistics are based on type III sums of squares.
*Differences are significant at α=0.05.

Table 2.3b. Summary statistics of within-species ANCOVA of $\delta^{13}C$

Species	Variable	F-statistic[2]	P-value	df
Cisco	lake	19.45	0.0001*	6,43
	wet mass[1]	0.81	0.3740	1,43
	lake*wet mass	0.96	0.4648	6,43
Emerald shiner	lake	5.23	0.0009*	5,39
	wet mass	17.92	0.0010*	1,39
	lake*wet mass	3.87	0.0061*	5,39
Rainbow smelt	lake	8.98	0.0001*	23,177
	wet mass	0.03	0.8585	1,177
	lake*wet mass	4.66	0.0001*	23,177
Spottail shiner	lake	106.51	0.2397	11,84
	wet mass	1.40	0.4144	1,84
	lake*wet mass	1.05	0.1145	11,84
Trout-perch	lake	58.92	0.0001*	15,127
	wet mass	0.36	0.5473	1,127
	lake*wet mass	0.49	0.9394	15,127
Yellow perch	lake	4.11	0.0001*	20,150
	wet mass	0.03	0.8725	1,150
	lake*wet mass	2.84	0.0001*	20,150

[1]Wet mass was $\log_e$ transformed prior to analysis.
[2]F-statistics are based on type III sums of squares.
*Differences are significant at α=0.05.

Table 2.4. Mean[1] trophic position (adj. $\delta^{15}N$) and $\delta^{13}C$ for all species captured

Species	Trophic position (‰)	Standard deviation	$\delta^{13}C$ (‰)	Standard deviation	N (number of lakes)
Alewife	3.06	2.89	-21.85	1.74	2
Bluegill	2.11	.	-27.95	.	1
Cisco	4.53	1.87	-28.25	1.60	7
Common shiner	4.29	0.91	-25.68	2.90	5
Emerald shiner	4.62	1.38	-26.24	1.54	6
Golden shiner	1.93	.	-27.57	.	1
Logperch	5.62	.	-25.28	.	1
Round goby	1.93	.	-18.78	.	1
Pumpkinseed	4.18	0.40	-24.42	2.44	3
Rainbow smelt	6.00	1.45	-26.86	2.16	24
Rock bass	5.00	.	-22.21	.	1
Slimy sculpin	5.79	1.04	-25.99	0.677	2
Spottail shiner	4.40	1.32	-24.40	3.18	12
Trout-perch	6.13	1.09	-25.97	2.28	16
Yellow perch	4.18	1.19	-24.16	3.07	20

[1]For species captured in more than one lake, means of lake-specific least-squared means were calculated (standardized mass of 8g). For species captured in only one lake, least-squared means (standardized mass of 8g) for that lake are presented.

Table 2.5. Summary statistics of species-specific linear regressions between trophic position and $\log_e$ wet mass and $\delta^{13}C$ and $\log_e$ wet mass[1]

	Trophic position			$\delta^{13}C$		
Species	**Slope**	**Intercept**	**P-value**	**Slope**	**Intercept**	**P-value**
Cisco	0.17	4.87	0.0429*	-0.04	-27.39	0.6950
Emerald shiner	0.10	4.44	0.6344	0.56	-27.64	0.0083*
Rainbow smelt	0.25	5.46	0.0001*	0.18	-27.24	0.0081*
Spottail shiner	0.61	3.11	0.0001*	0.23	-24.88	0.2895
Trout-perch	0.30	5.58	0.0011*	0.08	-26.13	0.5989
Yellow perch	0.28	3.65	0.0001*	0.26	-25.19	0.0025*

[1]Among-lake variation in trophic position and $\delta^{13}C$ was removed prior to analysis (see methods).

*Differences are significant at α=0.05.

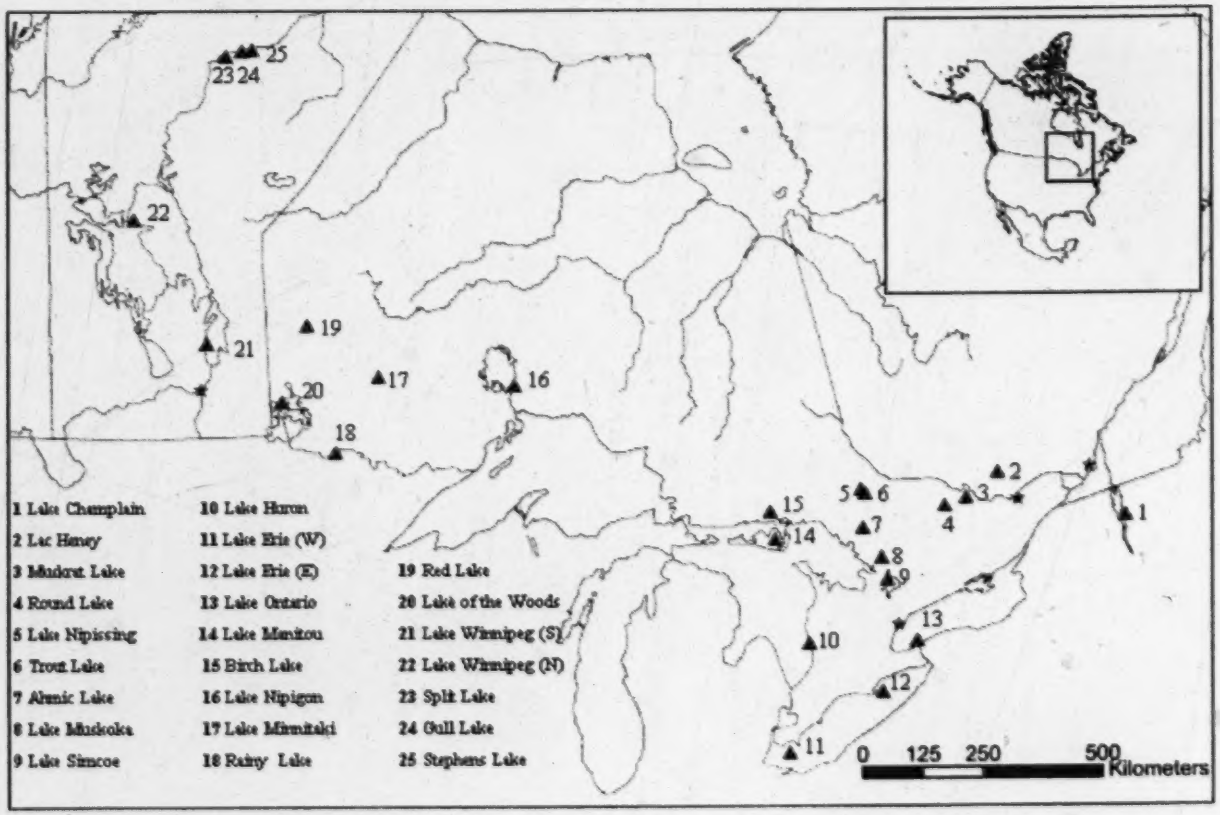

Figure 2.1. Map of the study lakes.

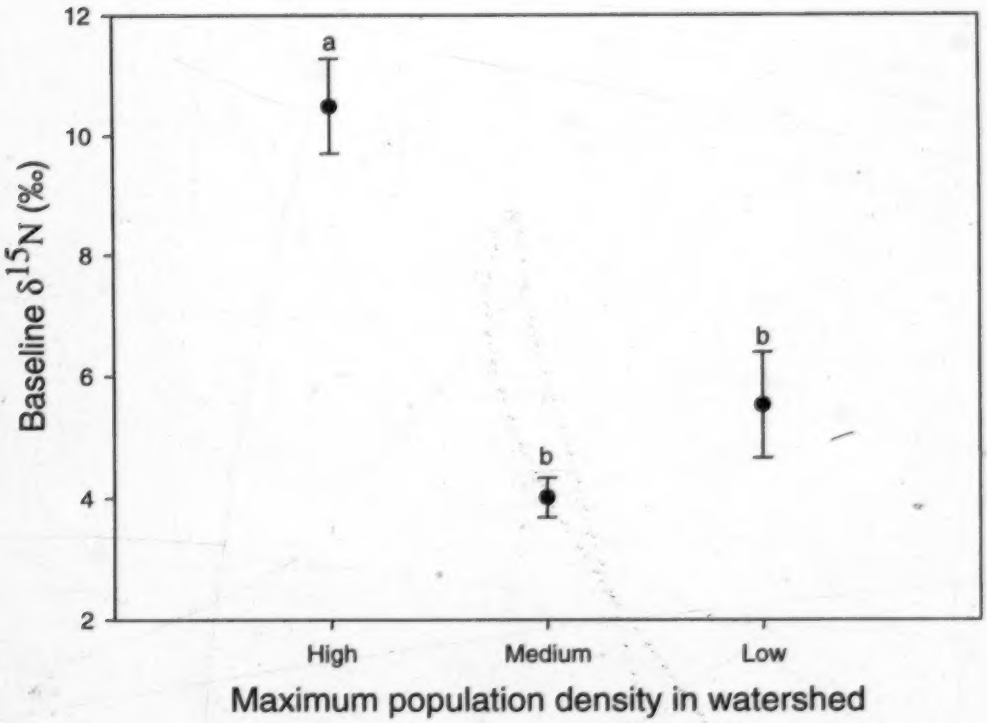

Figure 2.2. Mean ± SE baseline $\delta^{15}N$ among three classes of maximum human population density in the watershed, high (≥600 people/km^2), medium (10–599.99 people/km^2), and low (≤10 people/km^2). Lakes with high human population density (Lake Erie, Lake Ontario, and Lake Winnipeg (south basin)) had significantly higher baseline $\delta^{15}N$ than lakes with either medium or low maximum population density (Tukey's test, $P<0.05$). Letters indicate significant differences.

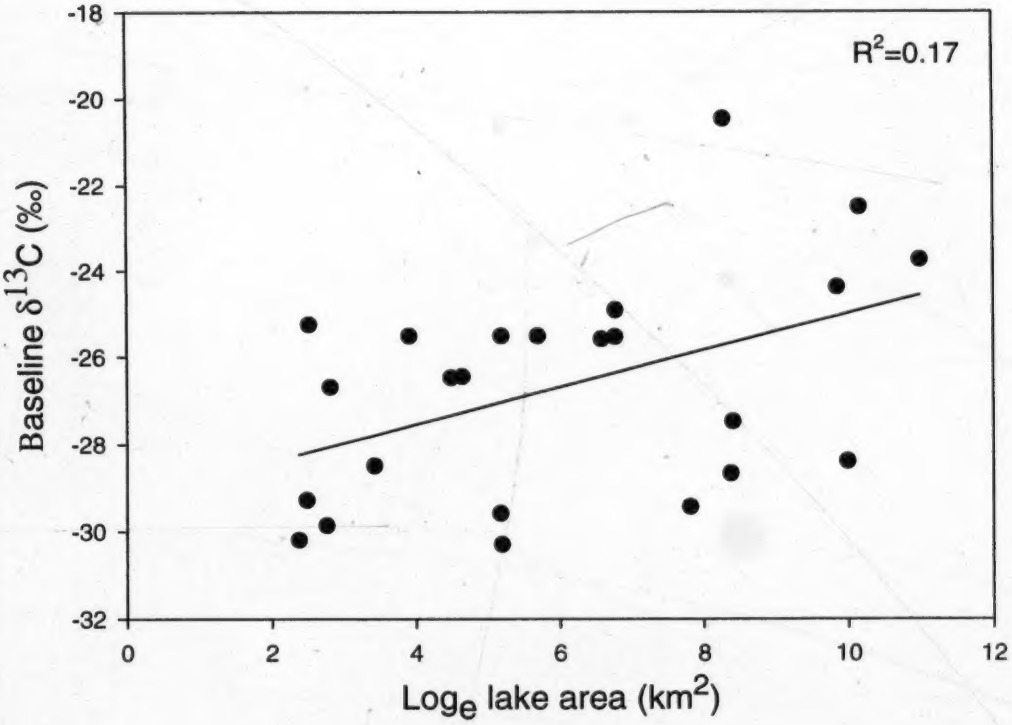

Figure 2.3. Linear regression of baseline $\delta^{13}C$ and $\log_e$ lake area. The relationship was significant and positive, baseline $\delta^{13}C$ increased with increasing lake area (Linear regression, R^2=0.17, P=0.0265, df=1,22).

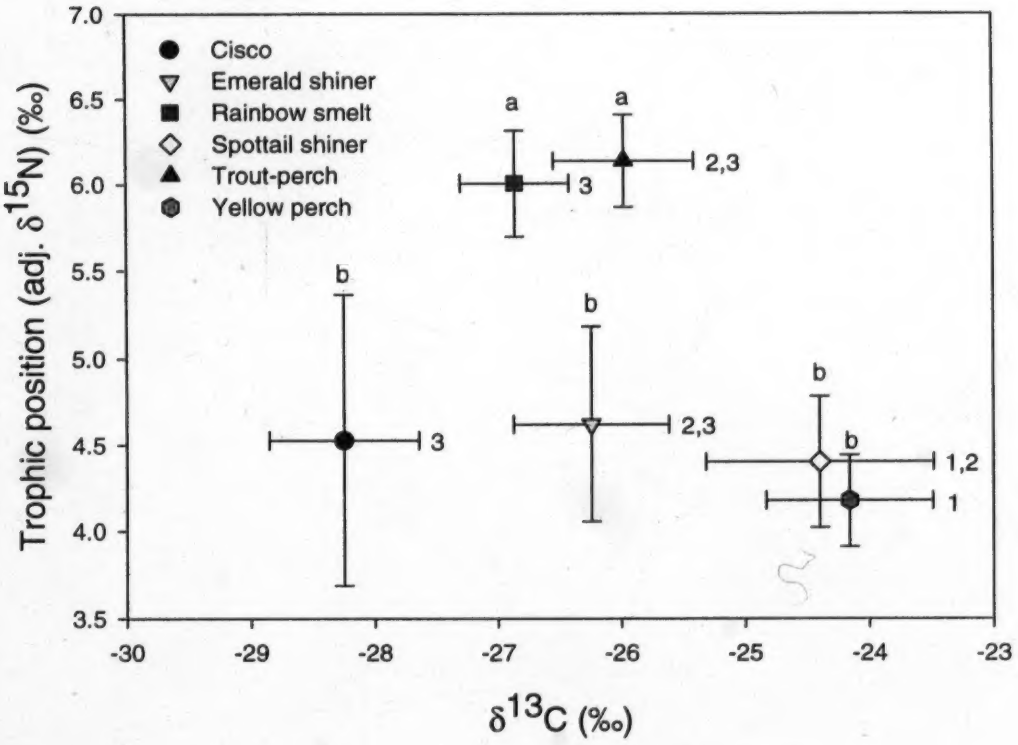

Figure 2.4. Mean ± SE trophic position and $\delta^{13}C$ for the six species analyzed statistically. Letters (a,b) indicate significant inter-species differences in trophic position and numbers (1,2,3) indicate significant inter-species differences in $\delta^{13}C$. At a standardized mass of 8 g, rainbow smelt and trout-perch had significantly higher trophic positions than all other species. Yellow perch had a significantly less negative $\delta^{13}C$ signature than all other species except spottail shiner, and spottail shiner had a significantly less negative $\delta^{13}C$ signature than either rainbow smelt or cisco (Tukey's test, $P<0.05$).

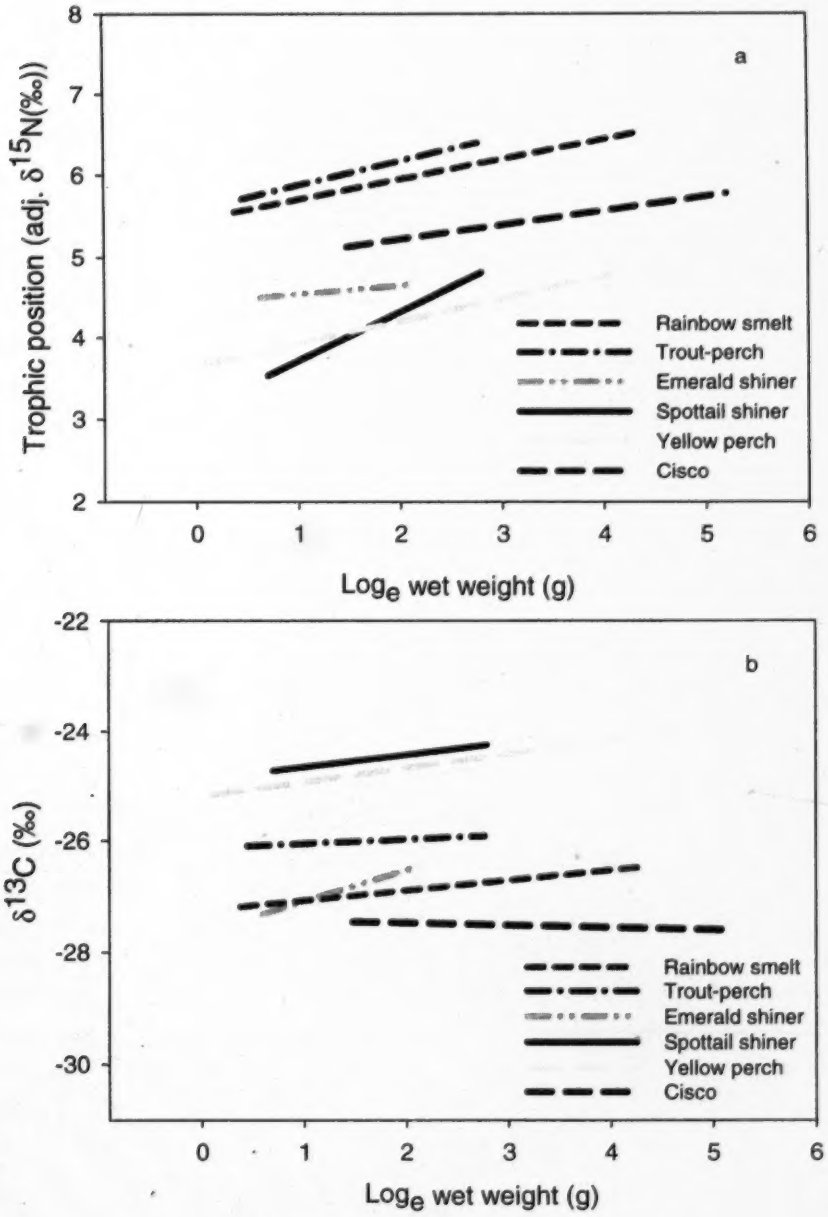

Figure 2.5a,b. Species-specific linear regressions of trophic position (a) and $\delta^{13}C$ (b) vs. log_e wet mass. Data used to obtain the relationships were corrected for among-lake differences but individual data points are not shown for clarity (N=765). Slopes were not significantly different among species for either trophic position (ANCOVA, F=1.92, P=0.11, df=5,722) or $\delta^{13}C$ (ANCOVA, F=1.46, P=0.20, df=5,765). The differences among species in trophic position and $\delta^{13}C$ are consistent across a range of body sizes.

Chapter 3: Forage fish growth rates and growth patterns

Introduction

Fish growth is indeterminate and flexible (Wootton 1990). If environmental conditions permit, iteroparous fish continue to grow after sexual maturity for the duration of their life span. Except for a brief period early in life, however, growth rates generally decrease with age and thus there is a theoretical maximum size (Mackay et al. 1990, Wootton 1990). The magnitude of maximum size and the rate of growth to maximum size differ among and within species, and can be affected by genetics, endogenous rhythms, temperature, photoperiod, salinity, oxygen availability, pH, prey availability, prey size, and prey type (see Wootton 1990, Jobling 1994, Boeuf and Le Bail 1999).

There are many ways to compare growth among species or populations of fish. Comparisons of mass-length regressions can identify differences in condition and growth patterns. The relationship between fish mass and length is curvilinear and can be represented by the following equation:

1. $W = aL^b$

where 'W' is mass, 'L' is length, and 'a' and 'b' are constants. If the relationship is log-transformed, $\log_e$'a' and 'b' are the intercept and slope, respectively, of the linear relationship (Mackay et al. 1990, Wootton 1990, Jobling 1994). The slope, 'b,' is the rate of change of mass with length and indicates how the shape of the fish changes with size. If the value of 'b' is approximately 3, growth is isometric and the shape of the fish does not change with increasing length. If 'b'<3 or 'b'>3, growth is allometric and the fish becomes lighter or heavier, respectively, with increasing length (Ricker 1979). Comparisons of 'b' (slopes) among species or populations of fish can therefore reflect differences in condition because fish that are heavier for their length are generally in better condition than those that are light for their length (Mackay et al. 1990, Wootton 1990). Fish condition is dependent on food size, quality, and availability, consumption rates, competition, and gonadal maturity. There are a number of different condition indices that can be calculated and compared

among populations or species, but covariance analysis of mass-length regressions is the preferred method (Wootton 1990).

Investigations of environmental, genetic, and social factors that affect fish growth often involve comparisons of growth rates among species or populations of fish. There are many different ways to calculate fish growth rate. The simplest, absolute growth rate, is the change in mass divided by time interval. Absolute growth rates vary with fish size, however, so relative growth rates are often used (Wootton 1990, Jobling 1994). Relative growth rates reflect the change in mass relative to the initial mass and are less sensitive to changes in fish size. When restricted to a short time interval, relative growth rates can be used to calculate instantaneous or specific growth rates (Wootton 1990, Jobling 1994). Although relative, instantaneous, and specific growth rates are better metrics of growth, they are not often practical for large-scale field studies. To calculate relative growth rates in the field, the study must be a mark-recapture design or there must be a reliable estimate of size-at-hatch.

Studies of fish growth are most commonly performed on commercially valuable species; much of the current research is focussed on aquaculture programs or sport-fish. The objectives of this study were to compare absolute growth rates and mass-length regressions among species and populations of forage fish and determine if among-population differences in growth could be attributed to latitude, conductivity, pH, maximum depth, or lake area.

Materials and Methods

Field Sampling, Sample Preparation, and Sample Analysis

Methods for field sampling of fish and collection of lake data were described in Chapter 2. A map of the study area and a table summarizing lake characteristics were also presented in Chapter 2 but are included in this chapter for convenience (Figure 3.1, Table 3.1).

Sagittal otoliths were removed from each fish during dissection for dorsal muscle tissue (Chapter 2). Ages were then determined by counting otolith annuli. For trout-perch, yellow perch, round goby, and alewife, whole otoliths were used for ageing (Nielsen and Johnson 1983). Otoliths from large rainbow smelt and cisco were processed using the "crack and burn" method. The otolith was broken in two at the

sulcus to produce a cross-section and burned lightly over a Bunsen burner flame (Nielsen and Johnson 1983). Spottail shiner, emerald shiner, and some smaller rainbow smelt and cisco otoliths were mounted in aruldite epoxy and sectioned transversely with a Buhler Isomet low-speed saw. The 0.5–1 mm sections were then polished using 600 grit sandpaper and 12 micron lapping film. Sectioned, cracked, and whole otoliths were cleared with glycerin before being viewed under a dissecting microscope. Two readers then counted otolith annuli and assigned ages to all fish. If the readings did not agree, a second method was used and the reading process was repeated. Age assignments were blind and based on assumed birth dates of January 1st (Nielsen and Johnson 1983). Once ages were assigned, absolute growth rates were determined for each fish by dividing the mass of the fish by the age in years.

A subsample of fish were aged using more than one otolith processing method to determine if there were method-specific biases. The mean agreement between methods was 84%. Of the remaining 16%, 8.5% of the ages assigned using the second method were higher and 7.5% were lower; no bias was apparent.

Statistical Analyses

As for stable isotopes, growth rates of rainbow smelt (*Osmerus mordax*), yellow perch (*Perca flavescens*), spottail shiner (*Notropis hudsonius*), emerald shiner (*Notropis atherinoides*), trout-perch (*Percopsis omiscomaycush*), and cisco (*Coregonus artedi*) were analyzed statistically. For the remaining species, there were either not enough lake replicates (golden shiner (*Notemigonus crysoleucas*), round goby (*Neogobius melanostomus*), slimy sculpin (*Cottus cognatus*), rock bass (*Ambloplites rupestris*), pumpkinseed (*Lepomis gibbosus*)) or concern that the species was not present in a random sub-sample of lakes (common shiner (*Luxilus cornutus*)). To enable general comparisons, however, mean growth rates were calculated for all species captured, including those excluded from subsequent statistical analysis.

Statistical analyses included linear regression (simple and stepwise multiple), analysis of covariance (ANCOVA), analysis of variance (ANOVA), and Pearson product-moment correlations (GLM procedure, REG procedure, CORR procedure, SAS Institute Inc., 1990). After each statistical analysis, residual plots were examined for homogeneity of variance and both Shapiro-Wilk and Kolmogorov-Smirnoff

statistics (tests of normality) were calculated and found to have P-values >0.05. To achieve homogeneity of variance, absolute growth rates, wet masses, and total lengths were $\log_e$ transformed prior to analyses.

After $\log_e$ –transformation, regressions of mass vs. length were performed for each species and the slopes ('b') were compared among species and among populations (within species) with analyses of covariance. Each lake was treated as a single population. To determine if differences among populations could be explained by lake characteristics, slopes for each species were related to pH, conductivity, lake area, latitude, longitude, and maximum depth with stepwise multiple regressions.

Analyses of covariance were also used to determine if there were species-specific differences in growth rate among lakes, and if growth rate was related to body size. These ANCOVAs were then used to standardize species-specific growth rates in each lake to a common fish size (see similar procedures in Chapters 2 and 4). The standardization was done to allow for comparisons of growth rate among populations or species where different size distributions of fish were captured; absolute growth rates are known to vary with fish size (Wootton 1990). Least-squared mean growth rates were calculated for each species in each lake at a mass of 8g. As discussed in Chapter 2, mass was chosen as the size covariate because mass data are less sensitive to differences in inter-species morphometry than length data. Eight grams was chosen as the size of standardization because it was within the range of captured fish for the majority of species in the majority of lakes. It was also close to the overall median size of fish captured and is a reasonable forage size for predators such as walleye (Knight and Vondracek 1993).

Once least-squared mean growth rates were calculated, they were compared among species with one-way ANOVA. Lake was included in the model as a block effect and a post-hoc Tukey's test was performed to identify significant pair-wise differences. Following this, an analysis was performed to assess whether changing the body size of comparison (8g) (within the range of captured body sizes common to all species) would affect the results of inter-species comparisons. Unlike similar analyses in Chapters 2 and 4, this could not be accomplished by regressing lake-corrected growth rate against $\log_e$ wet mass. This is because wet mass is part of the absolute

growth rate calculation and within an age class there is a 1:1 relationship between growth rate and wet mass. Among-lake differences in $\log_e$ wet mass were therefore determined and species-specific residuals from this model were plotted against age. Masses-at-age were then compared among species.

Finally, to determine if among-population (lake) differences in growth rate could be explained by environmental factors, species-specific least-squared mean growth rates were related to latitude, longitude, pH, lake area, and conductivity. This was done using Pearson product-moment correlations.

Results

Mass-length relationships

An analysis of covariance revealed that slopes of mass-length regressions ('b' values) did not differ significantly among species (ANCOVA, F=1.25, P=0.30, df=1,766). The slopes ranged from 2.98 (trout-perch) to 3.23 (emerald shiner), indicating that the growth of all species was close to isometric (Table 3.2). Within species, the slopes of mass-length relationships differed among populations for cisco, rainbow smelt, and yellow perch (ANCOVA, F>2.3, P<0.0024, df=6,43 (cisco), 23,177 (rainbow smelt), 20,161 (yellow perch)). These differences could not be explained by lake characteristics, however, as the slopes were not significantly related to latitude, longitude, maximum depth, conductivity, area, or pH (Stepwise multiple regression, R^2<0.15, P>0.05).

ANCOVA of absolute growth rate with lake and fish size

Growth rate differed significantly among lakes for all species (statistics in Table 3.3). For all species except cisco, there were significant and positive relationships between growth rate and $\log_e$ wet mass (statistics in Table 3.3). There was a significant interaction between lake and $\log_e$ wet mass in cisco, rainbow smelt, spottail shiner, and yellow perch (Table 3.3). For these species, the relationship between growth rate and $\log_e$ wet mass varied among lakes and analyses of growth rate among populations should only be made at a standardized fish mass.

Comparisons among species

Comparing all species captured, slimy sculpin, spottail shiner, and emerald shiner had the lowest mean growth rates while cisco and yellow perch had the highest mean growth rates (Table 3.4). The remainder of species had intermediate growth rates (Table 3.4). There were significant differences among the six species analyzed statistically (ANOVA, $F=29.72$, $P<0.0001$, $df=5,77$) (Figure 3.2). This analysis, which included species as a class variable and lake as a block effect, explained 64% of the variation in absolute growth rates ($R^2=0.64$). A post-hoc Tukey's test revealed that yellow perch and cisco had significantly higher growth rates than all other species (Tukey's test, $P<0.05$) and were not significantly different from each other (Tukey's test, $P>0.05$) (Figure 3.2). Trout-perch and rainbow smelt had significantly higher growth rates than emerald shiner (Tukey's test, $P<0.05$) but were not significantly different from each other or spottail shiner (Tukey's test, $P>0.05$).

Wet mass-age relationships

The slopes of $\log_e$ wet mass-age regressions differed significantly among species (ANCOVA, $F=19.13$, $P<0.0001$, $df=5,766$). Wet mass increased most rapidly with age in yellow perch (Figure 3.3; Table 3.5). Cisco and emerald shiner wet masses increased relatively slowly with age, and rainbow smelt, spottail shiner, and trout-perch were intermediate (Figure 3.3; Table 3.5). Despite these different slopes, however, only one pair-wise comparison of absolute growth rate changed with age. At ages below three, cisco had a higher mass-at-age (and therefore higher absolute growth rate because growth rate = mass/age) than yellow perch. Above age 3, however, yellow perch had higher growth rates than cisco. Within the range of ages common to all species (1-5), all other pair-wise comparisons of growth rate were consistent; yellow perch and cisco had higher growth rates than all other species, and emerald shiner and spottail shiner had lower growth rates than all other species. Because inter-species differences in absolute growth rate were consistent across the range of captured ages, they would also be consistent across the range of captured body sizes; fish that are larger at a given age are younger at a given size.

Growth rate and environmental factors

As mentioned above, growth rate differed among populations (lakes) for all species. Species-specific correlations of growth rate and lake characteristics showed that latitude and conductivity were most strongly related to growth rate. Yellow perch growth rates were significantly and negatively related to latitude and trout-perch growth rates were significantly and positively related to conductivity (Table 3.6). There were also marginally significant ($P<0.1$) relationships between rainbow smelt growth rate and conductivity (positive) and between spottail shiner growth rate and latitude (negative) (Table 3.6).

Discussion

Mass-length regressions

Growth patterns were close to isometric and were not significantly different among species. This means that the forage fish species in this study tend to retain their shape as they grow (Ricker 1979). Although there have been very few previous studies of forage fish growth patterns, the intercepts and slopes ('a' and 'b' values) obtained in this study agree reasonably well with previously published results (Table 3.2). For yellow perch and cisco, 'b' values obtained in this study were within the range of previously published findings (Carlander 1969, Brazo et al. 1975, Chadwick 1976, Froese and Pauly 2003). The values of 'b' for rainbow smelt, trout-perch, and emerald shiner were higher in this study than in previous studies, but many of the previous studies were conducted in the United States or exclusively in the Great Lakes and most were conducted over 30 years ago (Beckman 1942, Kinney 1950, Baten and Tack 1952, Swingle 1965, Froese and Pauly 2003). Temporal and spatial differences may thus explain the discrepancies. Literature values for spottail shiner were not found.

Differences among populations in mass-length slope could not be explained by latitude, longitude, pH, area, conductivity, or maximum depth. Previous studies have shown that rainbow smelt and yellow perch growth is dependent on prey size and prey type (Evans and Loftus 1987, Hayward and Margraf 1987, Boisclair and Leggett 1989). When larger, more energy-efficient prey are available, growth rates

increase. Mass-length slopes may therefore be most closely related to variables that reflect the size and type of available prey or density of competitors.

Growth rate comparisons among species and mass-age relationships

The inter-species differences in growth rate were expected and where data are available, correspond to differences in maximum size. Previous authors have shown that cisco, yellow perch, and rainbow smelt reach larger maximum sizes than emerald shiner (Froese and Pauly 2003). At a standardized mass of 8g, I found that cisco, yellow perch, and rainbow smelt had higher absolute growth rates than emerald shiner. These differences were consistent across the range of captured ages and would therefore be consistent across the range of captured body sizes.

Inter-species differences in forage fish growth rates and maximum size could affect contaminant concentrations and contaminant transfer to predator fish. At a given contaminant intake, species that grow faster should have lower bioaccumulative contaminant concentrations than species that grow more slowly. This is because fast-growing fish will be relatively young at a given size and younger fish will have shorter exposure histories than older fish (de Freitas et al. 1974, Huckabee et al. 1979). Also, fish with high growth rates often have high growth efficiencies. These fish may have lower contaminant concentrations because they produce a relatively large amount of flesh to dilute their contaminant burden (Meinertz 1995, Kidd et al. 1999, Essington and Houser 2003). Finally, food-web transfers of contaminants to predator species may be reduced when forage species reach large maximum sizes and become unavailable to gape-limited predators.

Growth rate and environmental factors

Species-specific growth rates differed significantly among populations and were most closely related to latitude and conductivity. In spottail shiner and yellow perch, there were negative relationships between growth rate and latitude. There are two possible reasons for this. The first is that higher latitude lakes are generally colder. Optimal temperatures for fish growth vary with consumption rate, but yellow perch and cyprinid species tend to prefer water temperatures >20°C (Jobling 1981, Hayward and Margraf 1987, Jobling 1994). Also, higher latitude lakes have shorter

growing seasons than lower latitude lakes. Further research that includes detailed lake temperature data and an analysis of growing degree-days would clarify which factor is most responsible for the negative relationships between growth rate and latitude.

Rainbow smelt and trout-perch growth rates were positively related to conductivity. This could be an indirect indication of pH and/or productivity effects on growth rate. In general, lakes in this study that had relatively high conductivity also had relatively high pH (the exception was Lake Simcoe) and primary productivity (assessed from literature sources and limited chlorophyll-*a* and total phosphorus data). Fish growth can be negatively affected by low pH because of decreased food availability and consumption and increased heavy metal toxicity (Fromm 1980, Pagenkopf 1983, see Wootton 1990). The lowest pH observed in this study (6.9), however, was above the range thought to cause chronic effects on fish populations (5.5-6.5) (Fromm 1980). As well, there was no direct relationship between rainbow smelt or trout-perch growth rates and pH. It is more likely, therefore, that the positive relationships between growth rate and conductivity were due to increased prey availability in lakes with higher conductivity and productivity.

Future research on among-population differences in forage fish growth should include an analysis of prey size, type, and availability. This may be particularly important for species that experience ontogenetic diet shifts to larger prey. Cisco, rainbow smelt, and yellow perch can become piscivorous at large sizes and yellow perch and rainbow smelt can switch from small to large zooplankton and from zooplanktivory to benthivory (Scott and Crossman 1973, Evans and Loftus 1987, Sherwood et al. 2002b). Diet switches to larger prey increase growth rates, growth efficiency, and maximum size because the metabolic cost of foraging decreases as the ratio of prey size to fish size increases (Pazzia et al. 2002, Sherwood et al. 2002a, Sherwood et al. 2002b). In the absence of acidification problems or other anthropogenic impacts, it is therefore likely that among-population differences in forage fish growth rate are more closely related to variables that describe food type, size, and availability than to variables that describe lake characteristics.

Conclusions

Based on the results of this study, it appears that growth patterns were similar among the forage species analyzed and nearly isometric. Growth rates and mass-age relationships differed among species, however. At a standardized body mass of 8g, cisco and yellow perch had the highest absolute growth rates and emerald shiner and spottail shiner had the lowest. Mass increased most rapidly with age in yellow perch and least rapidly in cisco and emerald shiner. Despite this, inter-species differences in growth rate were consistent across the range of captured ages (and therefore sizes) common to all species. Within species, growth rates were negatively related to latitude and positively related to conductivity. These relationships may be due to low temperatures and short growing seasons at higher latitudes, and higher prey availability in lakes with higher conductivity.

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Table 3.1. Summary of lake characteristics

Lake	Lat.	Long.	Conductivity (µS/cm)	Max. Depth (m)	Area (km^2)	pH	Invasion Year[1]
Ahmic	45°37'	79°42'	36	27	16	7.9	1981
Birch	46°18'	81°58'	26	27	11	7.5	1980
Champlain	44°30'	73°12'	220	129	1140	7.8	Native
Gull	56°19'	95°22'	98	.	70	8.0	1996
Heney	45°57'	75°57'	131	33	12	8.2	Native
Manitou	45°47'	82°00'	254	49	105	8.2	1950
Minnitaki	49°58'	92°00'	57	49	181	8.2	1991
Muskoka	45°00'	79°25'	46	67	90	7.9	1950
Muskrat	45°40'	76°55'	291	64	12	8.6	Native
Nipigon	49°50'	88°58'	134	137	4481	7.6	1976
Nipissing	46°19'	79°28'	69	52	873	7.8	1964
Rainy	48°36'	93°24'	55	49	881	8.4	1990
Red	51°03'	93°57'	57	43	177	8.1	1980
Round	45°38'	77°30'	58	55	31	7.9	1989
Simcoe	44°37'	79°25'	360	42	725	6.9	1960
Trout	46°20'	79°27'	93	68	16	7.8	1964
Winnipeg (N)	53°11'	99°16'	371	36	21925	8.6	1990
Winnipeg (S)	50°48'	96°59'	212	11	2475	.	1990
Lake of the Woods	49°43'	94°49'	.	66	4350	.	1990
Erie (E)	42°47'	80°12'	.	64	7375	.	1935
Erie (W)	41°50'	82°45'	.	10	3975	.	1935
Huron	43°45'	81°43'	.	228	59570	.	1931
Ontario	43°15'	79°04'	.	224	19009	.	1929
Split	56°08'	96°15'	191	.	280	8.0	1996
Stephens	56°23'	96°55'	201	.	299	8.0	1996

[1]Invasion year is the first report of rainbow smelt in a lake.

Table 3.2 Values of 'a' (intercept)[1] and 'b' (slope) for mass-length regressions

	Literature values		Present study	
Species	**a (intercept)**	**b (slope)**	**a (intercept)**	**b (slope)**
Cisco	0.0012-0.00488[2]	2.71-3.45[2]	0.00483	3.17
Emerald shiner	0.0195[3]	2.73[3]	0.00422	3.23
Rainbow smelt	0.0068-0.0252[4,5]	2.81-3.11[4,5]	0.03381	3.16
Spottail shiner	-	-	0.00635	3.15
Trout-perch	0.0093[6]	3.08[6]	0.00961	2.98
Yellow perch	0.0068-0.0316[7,8]	2.78-3.30[7,8]	0.00751	3.14

[1]Values of 'a' from $\log_c$-$\log_e$ relationships have been back-transformed.
[2]Carlander 1969; [3]Swingle 1965; [4]Baten and Tack 1952; [5]Beckman 1942; [6]Kinney 1950; [7]Brazo et al. 1975; [8]Chadwick 1976.

Table 3.3. Summary statistics of within-species ANCOVA of growth rate

Species	Variable	F-statistic[2]	P-value	df
Cisco	lake	4.35	0.0058*	4,35
	wet mass[1]	1.73	0.1972	4,35
	lake*wet mass	4.78	0.0020*	4,35
Emerald shiner	lake	7.96	0.0001*	5,39
	wet mass	49.0	0.0001*	1,39
	lake*wet mass	0.39	0.8560	5,39
Rainbow smelt	lake	4.57	0.0001*	22,175
	wet mass	75.6	0.0001*	1,175
	lake*wet mass	3.52	0.0001*	22,175
Spottail shiner	lake	1.91	0.0499*	11,84
	wet mass	61.7	0.0001*	1,84
	lake*wet mass	2.31	0.0159*	11,84
Trout-perch	lake	6.52	0.0001*	15,127
	wet mass	19.5	0.0001*	1,127
	lake*wet mass	1.51	0.1143	15,127
Yellow perch	lake	3.89	0.0001*	19,151
	wet mass	290	0.0001*	1,151
	lake*wet mass	3.07	0.0001*	19,151

[1]Wet mass was $\log_e$ transformed prior to analysis.
[2]F-statistics are based on type III sums of squares.
*Differences are significant at α=0.05.

Table 3.4 Mean[1] absolute growth rates for all species captured

Species	Mean[1] absolute growth rate (g/year)	Standard deviation	N (number of lakes)
Alewife	3.94	0.36	2
Bluegill	5.02	.	1
Cisco	7.29	0.66	7
Common shiner	3.31	0.31	5
Emerald shiner	2.32	0.24	6
Golden shiner	5.93	.	1
Round goby	3.63	.	1
Logperch	5.02	.	1
Pumpkinseed	6.17	0.13	3
Rainbow smelt	3.63	0.25	24
Rock bass	5.55	.	1
Slimy sculpin	2.10	0.21	2
Spottail shiner	2.79	0.16	12
Trout-perch	4.14	0.30	16
Yellow perch	7.31	0.31	20

[1]For species captured in more than one lake, means of lake-specific least-squared means were calculated (standardized mass of 8g). For species captured in only one lake, least-squared means (standardized mass of 8g) are presented for that lake.

Table 3.5. Summary statistics of intraspecific linear regressions between $\log_e$ wet mass and age[1]

Species	Slope	Intercept	P-value
Cisco	0.134	3.04	0.0001*
Emerald shiner	0.092	1.21	0.0423*
Rainbow smelt	0.363	1.40	0.0001*
Spottail shiner	0.281	1.17	0.0001*
Trout-perch	0.276	1.42	0.0001*
Yellow perch	0.527	1.87	0.0001*

[1]Among-lake variation in $\log_e$ wet mass was removed prior to analysis (see methods).
*Regressions are significant at α=0.05.

Table 3.6. Summary of Pearson product-moment correlations between intraspecific growth rates and lake characteristics[1]

Species	Variable	Correlation coefficient	P-value
Cisco	pH	0.59	0.410
Emerald shiner	maximum depth	0.43	0.473
Rainbow smelt	conductivity	0.42	0.083
Spottail shiner	latitude	-0.64	0.062
Trout-perch	conductivity	0.69	0.013*
Yellow perch	latitude	-0.52	0.025*

[1]The variables presented are those that were most closely related to intraspecific $\log_e$ growth rates. If nothing was significant, the variable with the lowest P-value is shown. All correlations are based on least-squared means calculated at a standardized mass of 8g.
*Relationships are significant at α=0.05.

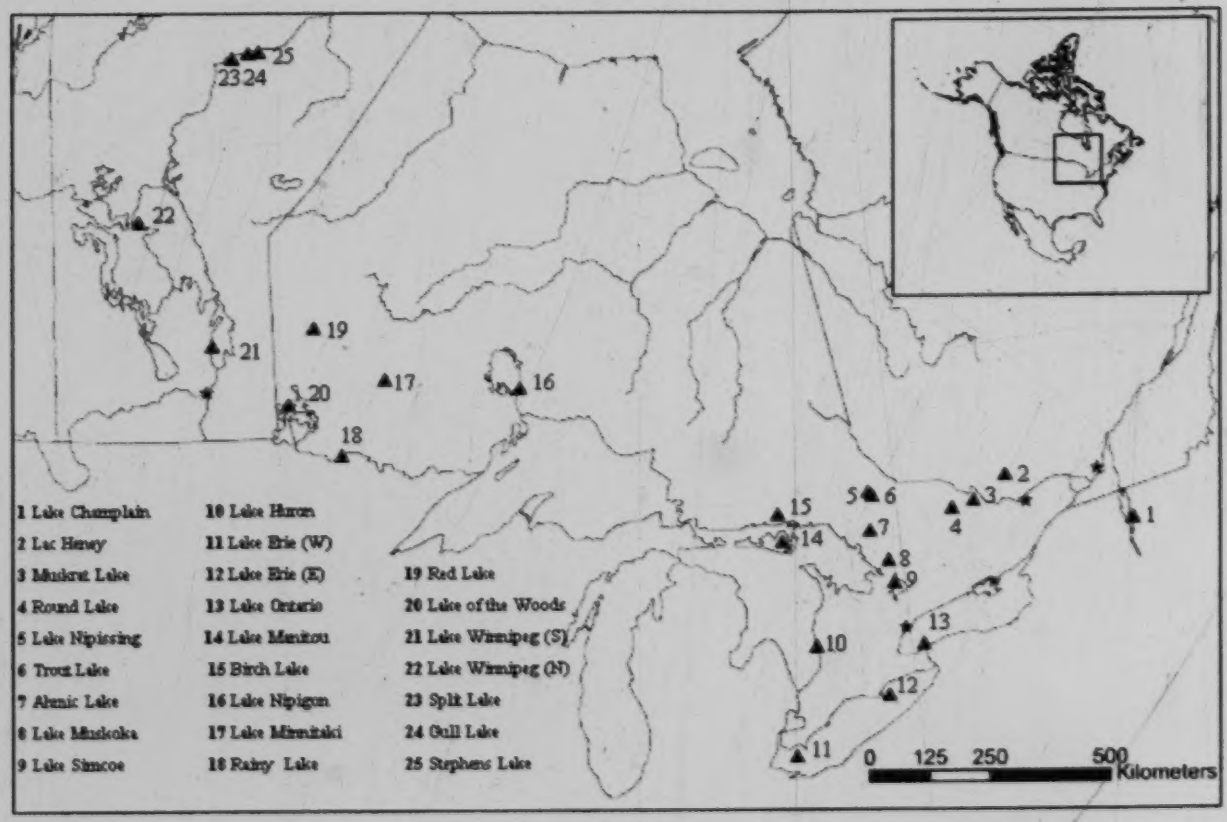

Figure 3.1. Map of the study lakes.

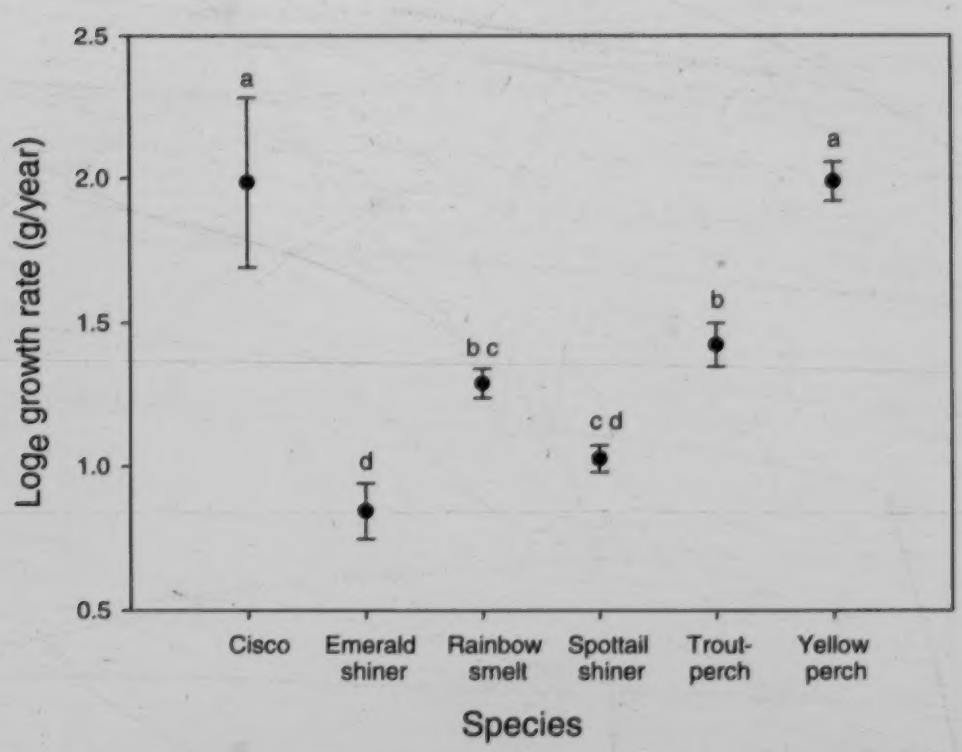

Figure 3.2. Least-squared mean (standardized mass=8 g) $\log_e$ growth rates $\pm$ SE. Yellow perch and cisco had significantly higher growth rates than all other species (Tukey's test, $P<0.05$) and emerald shiner had significantly lower growth rates than all other species except spottail shiner (Tukey's test, $P<0.05$). Letters indicate significant pair-wise differences.

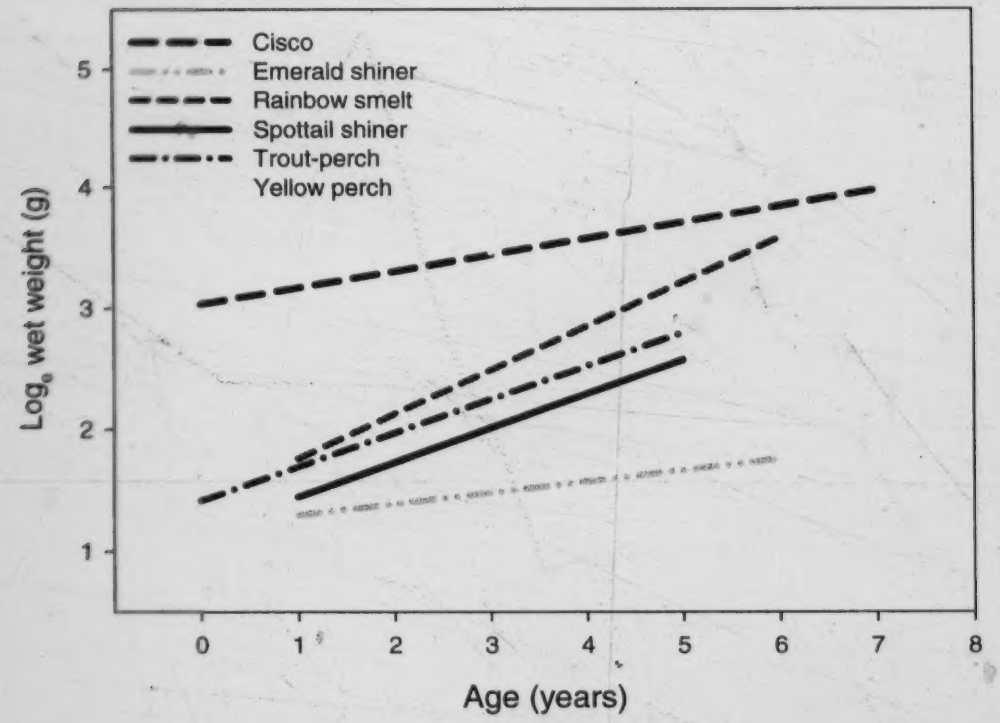

Figure 3.3. Species-specific linear regressions of $\log_e$wet mass vs. age. Data used to obtain the relationships were corrected for among-lake differences but individual data points are not shown for clarity (N=778). Slopes were significantly different among species (ANCOVA, F=19.13, P<0.0001, df=5,766). It does not appear, however, that changing the size of standardization would alter the inter-species comparison of growth rate (wet mass/age); cisco and yellow perch had higher mass-at-age than all other species across the range of captured ages and spottail shiner and emerald shiner had lower mass-at-age than all other species across the range of captured ages.

Chapter 4: Mercury dynamics in forage fish communities

Introduction

Mercury (Hg) is a neurotoxin and contaminant of concern for both ecological and human health reasons. It is derived from natural and anthropogenic sources and mercury inputs to lakes vary with geology, distance from anthropogenic sources, precipitation, the percentage and type of wetlands present, and drainage basin size, morphometry, and water yield (Jonasson and Boyle 1972, Rudd 1995, St. Louis et al. 1996, Trip and Allan 2000, Validya and Howell 2002). Once present in lake systems, inorganic Hg can be microbially converted to organic methylmercury (Jensen and Jernelov 1969). Methylmercury derived from the drainage basin, precipitation, and in-lake methylation processes can then biomagnify and bioaccumulate through aquatic food chains to top predator fish (Cabana and Rasmussen 1994, Kidd et al. 1995, Post 2002). Predators (including humans) that consume Hg-contaminated fish can experience adverse neurological and reproductive health effects (Harada 1976, Scheuhammer et al. 1998). The Canadian guideline for human fish consumption is 0.5 μg/g (IJC 1977). The flesh of large sport-fish species often exceeds these concentrations (Wren et al. 1991, Bodaly et al. 1993), and the risk to human health is particularly high in populations that rely on subsistence fisheries (Wheatley and Paradis 1995).

Fish Hg concentrations ([Hg]) vary with mercury inputs to lakes (discussed above), in-lake Hg methylation, direct uptake of Hg by fish, and biomagnification and bioaccumulation processes. Factors that affect in-lake methylation include dissolved organic carbon concentrations, pH, lake temperature, natural flooding, and reservoir development (Bodaly et al. 1984, Xun et al. 1987, Miskimmin et al. 1992, Bodaly et al. 1993, Kelly et al. 2003). Direct uptake of Hg by fish is affected by water hardness and pH; uptake is reduced when water hardness and pH are high (Pagenkopf 1983, Rodgers and Beamish 1983). Bioaccumulation and biomagnification processes in fish are influenced by age and size (Scott and Armstrong 1972, Allen-Gil et al. 1995, Power et al. 2002), food chain structure, trophic position (Cabana and Rasmussen 1994, Cabana et al. 1994, Kidd et al. 1995, Power et al. 2002), growth rate, and body condition (Suns and Hitchin 1990, Greenfield et al. 2001, Sonesten 2003).

Investigations of the effects of trophic position and food chain structure on [Hg] have become increasingly popular since the development of stable C and N ($\delta^{13}C$ and $\delta^{15}N$) isotope techniques (Peterson and Fry 1987). Most studies have found significant and positive relationships between $\delta^{15}N$ (an index of trophic position) and [Hg] (Cabana and Rasmussen 1994, Kidd et al. 1995, Power et al. 2002). These studies have always included, however, long-lived predator species such as lake trout (*Salvelinus namaycush*), northern pike (*Esox lucius*), walleye (*Sander vitreus*), or burbot (*Lota lota*). As well, although the relationships are often significant and positive among species, relationships within species are more variable (Kidd et al. 1995, Power et al. 2002).

A recent study found that body condition and pH were better predictors of yellow perch (*Perca flavescens*) [Hg] than $\delta^{15}N$-determined trophic position (Greenfield et al. 2001). Similarly, Swanson et al. (2003) found that $\delta^{15}N$- determined trophic position was not related to [Hg] in forage fish communities that had been invaded by rainbow smelt (*Osmerus mordax*). This was of considerable interest because rainbow smelt were trophically elevated relative to native forage fish species and were expected to cause an increase in predator fish [Hg] through food chain lengthening. In fact, predator fish [Hg] declined after smelt invasion (Johnston et al. 2003).

Previous authors have argued that longevity and growth rate may be as important in explaining fish [Hg] as biomagnification from prey (de Freitas et al. 1974, Huckabee et al. 1979). Swanson et al. (2003) hypothesized that the decoupling of forage fish [Hg] from trophic position was due to inter-species differences in growth rate. The relationship between growth rate and [Hg] is often inverse because fish that are younger at a given size have been exposed to Hg for a shorter time period (de Freitas et al. 1974, Huckabee et al. 1979). Also, at a given mercury intake, fish that are growing faster often have higher growth efficiencies and produce more flesh to dilute their Hg burden. This effect is called growth dilution, and it has been observed in many systems (de Freitas et al. 1974, Meinertz 1995, Rask et al. 1996, Kidd et al. 1999, Essington and Houser 2003). Growth dilution can also occur at the base of the food chain. In more productive lakes, faster growing phytoplankton cells

and higher phytoplankton biomass dilute Hg. This dilution effect can work through the food chain and result in lower fish [Hg] (Larsson et al. 2000, Pickhardt et al. 2002).

The objectives of this study were to determine: 1) relationships between fish [Hg] and size in a suite of forage fish species; 2) inter-species differences in [Hg]; and, 3) biotic ($\delta^{15}N$, $\delta^{13}C$, and growth rate) and abiotic (conductivity, pH, lake area, lake depth, latitude) factors that determine Hg concentrations in forage fish communities in central Canada.

Materials and Methods

Field Sampling, Sample Preparation, and Sample Analysis

Methods for fish sampling and dissection, growth rate determinations, stable C and N isotope ratio determinations, and collection of lake data were described in Chapters 2 and 3. A map of the study area and a table summarizing lake characteristics were presented in Chapters 2 and 3 but are also shown in this chapter for convenience (Figure 4.1, Table 4.1). Also, as described in Chapter 2, "trophic position" refers to $\delta^{15}N$ signatures that have been adjusted for baseline differences among lakes.

Methylmercury is the form of mercury that biomagnifies and bioaccumulates in food chains. In fish, the vast majority (90–100%) of total mercury is comprised of methylmercury (Grieb et al. 1990, Allen-Gil et al. 1995, Becker and Bigham 1995). Measurements of total mercury thus represent an inexpensive proxy for methylmercury. Freeze-dried and pulverized fish muscle samples were analyzed for total flesh mercury concentration at the Freshwater Institute Metals Lab using a modified hot block method followed by cold vapour atomic absorption spectroscopy (Hendzel and Jamieson 1976). Dry tissue (0.04 g) was digested with 5 mL of 4:1 trace-metal-analysis-grade nitric: sulfuric acid at 120°C for 3 hours and 180°C for 5 hours. It was then cooled and diluted to 25 mL of distilled, de-ionized water. Elemental Hg was released from solution with chloride reductant before being analyzed in an LDS model 3200 Mercury Monitor. All total mercury concentrations are reported in μg/g dry mass. The mean difference ± SD between randomly selected duplicate sub-samples was 0.03 ± 0.06 μg/g.

Statistical Analysis

Similar to the stable isotope and growth rate analyses, only 6 species were analyzed statistically for mercury concentration. These included rainbow smelt, yellow perch, spottail shiner (*Notropis hudsonius*), emerald shiner (*Notropis atherinoides*), trout-perch (*Percopsis omiscomaycush*), and cisco (*Coregonus artedi*). To enable general comparisons, however, means were calculated for all species, including those excluded from subsequent statistical analysis.

Statistical analyses on the 6 selected species included linear regression (simple and stepwise multiple), analysis of covariance (ANCOVA), analysis of variance (ANOVA), and Pearson product-moment correlations (GLM procedure, REG procedure, CORR procedure, SAS Institute Inc., 1990). Wet mass was $\log_e$ (ln) transformed prior to analysis. When more than one variable was significant in a stepwise multiple regression, part and partial correlations and collinearity diagnostics were calculated and examined. Variables with a moderate collinearity problem (condition index = 15-30) are identified in the results section (see below) but were retained in the model. Variables with a serious collinearity problem (condition index > 30) were deleted from the model.

Analyses of covariance were used to determine if there were species-specific differences in [Hg] among lakes, and if [Hg] was related to body size. They were also used to standardize [Hg] to a common fish body size (see similar procedures in Chapters 2 and 3). This standardization was done to allow for comparisons among populations or species where different size distributions of fish were captured. The most linear [Hg]-fish size relationship was between [Hg] and $\log_e$ wet mass. The ANCOVA model therefore contained lake as the categorical variable and $\log_e$ wet mass as the continuous variable. Least-squared means were calculated for each species in each lake at a mass of 8g. Mass was chosen as the size covariate because it is less sensitive to differences in inter-species morphometry than length. Eight grams was chosen as the size of standardization because it was within the range of captured fish in most lakes and was close to the overall median size captured. Also, 8g is a reasonable prey size for predators such as walleye (Knight and Vondracek 1993). In

all of the following statistical procedures, analyses were performed on $\log_e$-transformed least-squared means at 8g.

Once calculated and transformed, least-squared mean Hg concentrations were compared among species with one-way ANOVA. Lake was included in the model as a block effect and a post-hoc Tukey's test was performed to identify significant pair-wise differences. Following this, two sets of analyses were performed. The first analysis (biotic analysis) examined the relationship between total [Hg] and biotic variables (growth rate, trophic position (adjusted δ^{15}), and $\delta^{13}C$). The second analysis (abiotic analysis) examined the relationship between total [Hg] and an array of abiotic variables. The abiotic variables included latitude, area ($\log_e$ transformed), specific conductivity, maximum depth, and pH. The Great Lakes were not included in the abiotic analyses because abiotic data were often missing and because there are historical and contemporary point sources of Hg on the Great Lakes.

The abiotic and biotic analyses were conducted both interspecifically and intraspecifically. Interspecific analyses were performed on all species pooled using stepwise multiple regression. Intraspecific relationships were examined with Pearson product-moment correlations.

Ideally, the abiotic and biotic analyses would have been combined. They were performed separately because complete abiotic data were only available for a subset (18/25) of the lakes sampled for fish. A final model is presented, however, that combines abiotic and biotic variables using stepwise multiple regression.

After each statistical analysis, residual plots were examined for homogeneity of variance and both Shapiro-Wilk and Kolmogorov-Smirnoff statistics (tests of normality) were calculated and found to have P-values >0.05. As well, an analysis was performed to further investigate the effect of body size on [Hg], and to assess if changing the body size of comparison (8g) (within the range of captured body sizes common to all species) would affect the observed results. To do this, among-lake differences in [Hg] were determined for each species with one-way ANOVA. Species-specific residuals from this model were then plotted against $\log_e$ wet mass and slopes and intercepts of the relationships were compared among species.

Results

ANCOVA of [Hg] with lake and fish size

An analysis of covariance revealed that in all species except spottail shiner there were significant differences in [Hg] among lakes (statistics in Table 4.2). In all species except trout-perch, there were also significant positive relationships between [Hg] and $\log_e$ wet mass (Table 4.2). In four species, rainbow smelt, spottail shiner, trout-perch, and yellow perch, there was a significant interaction between lake and $\log_e$ wet mass (Table 4.2). For these species, the relationship between total [Hg] and $\log_e$ wet mass varied among lakes and analyses among populations should only be made at a standardized fish mass.

Comparisons among species and range of values

If all 977 fish are considered, total [Hg] ranged from 0.05 µg/g (rainbow smelt and round goby in the Great Lakes) to 1.81 µg/g (emerald shiner in Lake Minnitaki). Emerald shiner had the highest mean total Hg concentration (0.86 µg/g), followed by spottail shiner (0.44 µg/g) (Table 4.3). All other species were in the range of 0.2–0.4 µg/g except for round goby, which had an extremely low mean Hg concentration (0.07 µg/g). It should also be noted that the other two shiner species, common shiner and golden shiner, had higher [Hg] than other species in the 0.2–0.4 µg/g range.

Least-squared mean total [Hg] varied significantly among the six species analyzed statistically (ANOVA, $F=11.84$, $P<0.0001$, $df=5,53$) (Figure 4.2). This model, which included species as a class variable and lake as a block effect, explained 74% of the variation in [Hg] ($R^2=0.74$). A post-hoc Tukey's test revealed that emerald shiner had significantly higher [Hg] than all other species and spottail shiner had significantly higher [Hg] than cisco, rainbow smelt, trout-perch, and yellow perch (Tukey's test, $P<0.05$). Cisco, rainbow smelt, trout-perch, and yellow perch were not significantly different from one another (Tukey's test, $P>0.05$) (Figure 4.2).

Hg concentration-fish size relationships

To determine if changing the body size of comparison (8g) would alter the observed differences in [Hg] among species, regressions of total [Hg] vs. $\log_e$ wet mass were computed and compared among species (the lake effect was first

removed). Emerald shiner had higher [Hg] than all other species across the range of captured body sizes common to all species. The only pair-wise comparisons that changed with body size were those that involved cisco. At low body sizes, cisco [Hg] was approximately equal to yellow perch, trout-perch, and rainbow smelt [Hg]. At higher body sizes, however, cisco had lower [Hg] than all other species (Figure 4.3).

The slopes of the relationships differed significantly among species (ANCOVA, F=3.78, P=0.0022, df=5,741). This difference appears to lie between cisco and all other species (Table 4.4) (Figure 4.3). Slopes for emerald shiner, rainbow smelt, spottail shiner, trout-perch, and yellow perch ranged from 0.085 to 0.13. Emerald shiner had the steepest slope (0.13). In contrast, the slope for cisco was 0.013. It is therefore evident that the increase in [Hg] with body size was less rapid for cisco than for any other species (Figure 4.3).

Biotic Analyses

When all species were pooled and [Hg] was regressed against trophic position, $\delta^{13}C$, and growth rate, all three variables were significant and negatively related to total [Hg] (Multiple regression, $R^2_{adj}=0.29$, $P<0.0001$, df=3,77). Part and partial correlations and change statistics revealed, however, that of the 29% of variation accounted for by this model ($R^2_{adj}=0.29$) growth rate was responsible for 22%. $\delta^{13}C$ and trophic position each accounted for approximately 3% of the variation. The relative strength of the relationships is illustrated in plots of [Hg] vs. growth rate, [Hg] vs. $\delta^{13}C$, and [Hg] vs. trophic position (Figure 4.4 a,b,c). There was also a moderate collinearity problem between $\delta^{13}C$ and the intercept (condition index=28) in the biotic model; this is another indication that $\delta^{13}C$ did not explain much variation in [Hg].

Relationships between total [Hg] and the biotic variables (trophic position, $\delta^{13}C$, and growth rate) were examined in each species separately with Pearson product-moment correlations. In yellow perch and rainbow smelt, there were significant negative relationships between [Hg] and growth rate, and in rainbow smelt there was also a significant negative relationship between [Hg] and $\delta^{13}C$. Trout-perch showed a marginal (P=0.078) negative relationship between [Hg] and growth rate.

The results of the intraspecific analyses are summarized in Table 4.5. Where there were no significant relationships, the variable with the lowest P value is reported.

Abiotic Analyses

When all species were pooled and least-squared mean [Hg] was regressed against pH, latitude, area ($\log_e$-transformed), maximum depth, and conductivity, a significant negative relationship was found between total [Hg] and conductivity (Multiple regression, R^2_{adj}= 0.25, P<0.0001, df=1,63). This relationship was also seen in four of the six species-specific correlations. Emerald shiner, rainbow smelt, trout-perch, and yellow perch [Hg] were significantly and negatively correlated with conductivity (r<-0.57, P<0.027) (Table 4.6). The relationship between spottail shiner [Hg] and conductivity was also negative and was close to significant (r=-0.69, P=0.0556). Other significant results in the intraspecific abiotic correlations included negative relationships between trout-perch total [Hg] and pH, and emerald shiner total [Hg] and area (Table 4.6).

Combined Biotic and Abiotic Analyses

A stepwise multiple linear regression revealed that when all species were pooled and all biotic and abiotic variables combined, growth rate and conductivity were significantly related to total [Hg] (Multiple linear regression, R^2_{adj}= 0.42, P<0.0001, df=2,55). Both of these relationships were negative and together explained 42% of the variation in [Hg]. Of this 42%, conductivity accounted for 26% and growth rate accounted for 16%. The strongest model for total [Hg], however, included growth rate, conductivity, and a species class variable. All three of these variables were significant (ANCOVA, F>4.4, P<0.04, df=1,56, 1,56, and 5,56), and together explained 60% of the variation in forage fish [Hg]. Neither of the interaction terms (species*growth rate or species*conductivity) were significant (P>0.05), meaning that the slopes of the [Hg]-growth rate and [Hg]-conductivity relationships were not significantly different among species. Because growth rate was also significantly related to conductivity (see Chapter 3), however, it was important to verify whether growth rate had an effect on total [Hg] that was independent of conductivity. To test this, I followed the example of Greenfield et al. (2001), and examined the relationship

between total [Hg] and growth rate within lakes (where conductivity is constant). Growth rate remained significant (ANCOVA, F=8.47, P=0.0053, df=1,51) and so is interpreted to have a significant and independent effect on total [Hg].

Discussion

Comparisons among species and range of values

Differences in [Hg] among species were consistent with previous findings. Swanson et al. (2003) found that spottail shiner had significantly higher total [Hg] than trout-perch, yellow perch, rainbow smelt, or cisco in 10 northwestern Ontario lakes. The present study confirmed these results over a much larger geographic range and with a slightly different array of species. At a standardized body mass of 8g, I found that emerald shiner and spottail shiner had significantly higher flesh Hg concentrations than trout-perch, yellow perch, rainbow smelt, or cisco.

When converted to wet weight concentrations, many individual emerald shiners and spottail shiners had total [Hg] (0.4-0.47 μg/g) that approached the Canadian human consumption guideline of 0.5 μg/g. This result is striking given that this is a forage rather than a predator species. Fish Hg concentrations in this range also pose health risks to fish-eating birds; 0.3-0.4 μg/g (wet) can cause impaired reproductive function in common loons (*Gavia immer*) (Barr 1986, Scheuhammer et al. 1998).

The low total [Hg] in round goby is an interesting finding. Round goby is a species that has recently invaded the Great Lakes region and is known to feed on zebra mussels (*Dreissena polymorpha*) in some areas (Ray and Corkum 1997, Diggins et al. 2002). One study showed that more than 50% of round goby diet can be composed of zebra mussels (Ray and Corkum 1997). Zebra mussels, as a primary consumer, should have relatively low [Hg]. This could explain the low [Hg] observed in round goby but further research would be required to confirm this.

Hg concentration-fish size relationships

Slopes of [Hg]-body size relationships differed among species, but it appears that changing the body size of comparison would not alter the ranking of species' [Hg]. Emerald shiner had higher [Hg] than other forage species across the range of

captured body sizes. It should be noted however, that the only species represented at wet mass >20g were cisco, rainbow smelt, and yellow perch. This has implications for predicting predator [Hg]. As mentioned by Swanson et al. (2003), it appears that to predict the magnitude and direction of [Hg] changes in predator species following rainbow smelt invasion, it is necessary to know both the species and size composition of the pre- and post-invasion diet. From this study, it appears that predator [Hg] would only increase post-invasion if the diet switch was from cisco to rainbow smelt The most likely predator fish to experience this diet shift is lake trout because it is a species that feeds almost exclusively on pelagic prey items.

Abiotic, biotic, and combined analyses of Hg concentration

The results of the biotic, abiotic, and combined analyses revealed that forage fish Hg concentrations were best explained by a species class variable, growth rate, and conductivity. Trophic position and $\delta^{13}C$ were significant variables in the biotic model only, and even then explained very little variation.

Many previous studies have found significant and positive relationships between trophic position and [Hg] (Cabana and Rasmussen 1994, Kidd et al. 1995, Power et al. 2002). As mentioned above, however, these studies have always included large, long-lived predatory species. It is possible that when examining the relationship between trophic position and [Hg] within a trophic guild (i.e., forage fish) of relatively short-lived fishes, the scale of trophic differentiation and the time for mercury exposure does not allow for a significant biomagnification effect. Other authors have made similar suggestions, reporting that short life span and opportunistic feeding between benthic and pelagic food sources (such as that displayed by many forage fishes) may reduce the potential for biomagnification (de Freitas et al. 1974, Huckabee et al. 1979, Cabana et al. 1994, Greenfield et al. 2001).

In this study, the [Hg]-trophic position relationship was negative. This is most likely an artefact of the species assemblage that was analyzed. The relationship was driven by the contrast of emerald shiners, which had higher [Hg] than all other forage species but a relatively low trophic position, to rainbow smelt, which occupied a high trophic position but had relatively low [Hg]. I suggest that if this study had included a

more complete forage fish species assemblage, the slope of the [Hg]-trophic position relationship would approach zero.

The [Hg]-$\delta^{13}C$ relationship was also significant and negative in the interspecific biotic model. Although there was a moderate collinearity problem with the intercept and the results should thus be interpreted with caution, similar relationships have been found in previous studies (Power et al. 2002). The reasoning for this is that benthic invertebrates at the base of the littoral food chain often have lower [Hg] than pelagic zooplankton (Tremblay 1999). This is also the reason why organisms that feed opportunistically in both the benthic and pelagic food chains have weak trophic position-[Hg] relationships; they may be feeding on prey that have similar trophic positions but different [Hg].

Growth rate was the best predictor of Hg concentration in the biotic model and was retained in the combined models. The relationship, as expected, was negative; fish that grew more slowly and were older at the standardized size of 8g had higher [Hg] than fish that grew faster and were younger at 8g. This may be because the fast-growing, younger fish had shorter exposure histories to Hg. Also, Hg biomagnification in fast-growing fish may be relatively low because of growth dilution effects. To confirm a growth dilution effect, however, data on forage fish growth efficiencies and prey [Hg] would be required.

Conductivity was the only significant abiotic predictor of interspecific fish [Hg] and was a slightly stronger predictor of [Hg] than growth rate in the combined model. It was also significant in four of six intraspecific analyses. The relationship between [Hg] and conductivity was always negative. There are three possible reasons for this. First, the drainage basins of the study lakes included in the abiotic and combined analyses were either totally within or partially within the Canadian Shield. Lakes in this region show strong positive relationships between conductivity and the concentration of major cations (either Ca^{2+} or Mg^{2+}) (Armstrong and Schindler 1971). Higher concentrations of these cations tend to decrease uptake and toxicity of metals such as mercury (Pagenkopf 1983, Rodgers and Beamish 1983). This is because Ca^{2+} and Mg^{2+} can compete with mercury for uptake sites on the gills of fishes (Pagenkopf 1983) and alter gill permeability and electric charge so as to inhibit cation (such as

CH_3Hg^+) uptake (McWilliams and Potts 1978, Rodgers and Beamish 1983). This is one of the reasons why many previous studies have found inverse relationships between fish [Hg] and water hardness (Hakanson 1988, McMurtry et al. 1989, Wren et al. 1991).

The negative relationship between [Hg] and conductivity may also be explained by correlations between conductivity and pH, and between conductivity and lake productivity. Lakes in this study that had relatively high conductivity also had relatively high pH (the exception was Lake Simcoe) and primary productivity (assessed from literature sources and limited chlorophyll a and total phosphorus data). Fish Hg concentrations tend to decrease with both increasing pH (McMurtry et al. 1989, Grieb et al. 1990, Greenfield et al. 2001) and increasing primary productivity (Kidd et al. 1999, Essington and Houser 2003). The relationship between [Hg] and pH is negative because low pH increases the bioavailability and bacterial uptake of Hg(II) for methylation (Miskimmin et al. 1992, Kelly et al. 2003). This results in increased methylation rates and net methylmercury production (Xun et al. 1987). Also, low pH increases direct uptake of Hg across fish gills (Ponce and Bloom 1991) and favours the production of mono-methylmercury (the more readily bioaccumulated and biomagnified form of Hg) over di-methylmercury (Wood 1980). These mechanisms may also explain the significant and negative intraspecific correlation found between [Hg] and pH in trout-perch.

Fish [Hg] decrease with increasing primary productivity for a number of reasons. First, fish growth rates tend to increase with primary productivity. It was demonstrated above, however, that there were independent effects of conductivity and growth rate on fish [Hg]. Another possible reason is that high primary productivity dilutes Hg at the base of the food chain, in algal cells. In more eutrophic systems, the burden of bioavailable Hg in the water column is distributed among a greater number of algal cells and those cells are growing quickly (Larsson et al. 2000, Pickhardt et al. 2002). These processes are referred to as bloom dilution and growth dilution, respectively, and have been shown to lower contaminant concentrations in zooplankton and fish. As well, higher sedimentation rates in more eutrophic systems

may increase the flux of contaminants out of the water column and into the sediment (Larsson et al. 1992, Larsson et al. 2000).

One other abiotic parameter was significantly related to fish [Hg]; emerald shiner [Hg] was significantly and negatively related to lake area. Bodaly et al. (1993) found a negative relationship between lake size and [Hg] in four fish species and attributed the relationship to differences in epilimnetic water temperatures. They found that the high epilimnetic water temperatures in smaller lakes increased the ratio of Hg methylation to demethylation. Temperature data for the study lakes would be required to confirm this hypothesis for emerald shiner.

Conclusions

The results of this research indicate that biomagnification processes are not readily discernible within a community of relatively short-lived, trophically similar organisms. Forage fish Hg concentrations were best predicted by growth rate and conductivity, not trophic position. Further research on a larger number of lakes and a wider array of water chemistry parameters would help clarify the relative influence of abiotic and biotic factors on Hg concentrations in forage fish communities. It would also be interesting to expand this research to include other contaminants, such as persistent organic pollutants.

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Table 4.1. Summary of lake characteristics

Lake	Lat.	Long.	Conductivity (µS/cm)	Max. Depth (m)	Area (km^2)	pH	Invasion Year[1]
Ahmic	45°37'	79°42'	36	27	16	7.9	1981
Birch	46°18'	81°58'	26	27	11	7.5	1980
Champlain	44°30'	73°12'	220	129	1140	7.8	Native
Gull	56°19'	95°22'	98	.	70	8.0	1996
Heney	45°57'	75°57'	131	33	12	8.2	Native
Manitou	45°47'	82°00'	254	49	105	8.2	1950
Minnitaki	49°58'	92°00'	57	49	181	8.2	1991
Muskoka	45°00'	79°25'	46	67	90	7.9	1950
Muskrat	45°40'	76°55'	291	64	12	8.6	Native
Nipigon	49°50'	88°58'	134	137	4481	7.6	1976
Nipissing	46°19'	79°28'	69	52	873	7.8	1964
Rainy	48°36'	93°24'	55	49	881	8.4	1990
Red	51°03'	93°57'	57	43	177	8.1	1980
Round	45°38'	77°30'	58	55	31	7.9	1989
Simcoe	44°37'	79°25'	360	42	725	6.9	1960
Trout	46°20'	79°27'	93	68	16	7.8	1964
Winnipeg (N)	53°11'	99°16'	371	36	21925	8.6	1990
Winnipeg (S)	50°48'	96°59'	212	11	2475	.	1990
Lake of the Woods	49°43'	94°49'	.	66	4350	.	1990
Erie (E)	42°47'	80°12'	.	64	7375	.	1935
Erie (W)	41°50'	82°45'	.	10	3975	.	1935
Huron	43°45'	81°43'	.	228	59570	.	1931
Ontario	43°15'	79°04'	.	224	19009	.	1929
Split	56°08'	96°15'	191	.	280	8.0	1996
Stephens	56°23'	96°55'	201	.	299	8.0	1996

[1]Invasion year is the first report of rainbow smelt in a lake.

Table 4.2. Summary statistics of within-species ANCOVA of [Hg]

Species	Variable	F-statistic[2]	P-value	df
Cisco	lake	22.78	0.0001*	5,43
	wet mass[1]	8.18	0.0065*	1,43
	lake*wet mass	1.12	0.3639	5,43
Emerald shiner	lake	27.27	0.0001*	5,38
	wet mass	15.23	0.0003*	1,38
	lake*wet mass	1.8	0.1359	5,38
Rainbow smelt	lake	2.6	0.0003*	22,174
	wet mass	26.02	0.0001*	1,174
	lake*wet mass	5.32	0.0001*	22,174
Spottail shiner	lake	1.71	0.0861	11,82
	wet mass	15.51	0.0002*	1,82
	lake*wet mass	1.5	0.0450*	11,82
Trout-perch	lake	1.81	0.0426*	15,109
	wet mass	2.91	0.0908	1,109
	lake*wet mass	5.56	0.0001*	15,109
Yellow perch	lake	2.86	0.0002*	19,146
	wet mass	32.55	0.0001*	1,146
	lake*wet mass	4.41	0.0001*	19,146

[1]Wet mass was $\log_e$ transformed prior to analysis.
[2]F-statistics are based on type III sums of squares.
*Differences are significant at α=0.05.

Table 4.3. Mean[1] total Hg concentrations for all species captured

Species	Mean[1] total Hg concentration (µg/g dry mass)	Standard deviation	N (number of lakes)
Alewife	0.20	0.27	2
Bluegill	0.26	.	1
Cisco	0.26	0.23	7
Common shiner	0.37	0.13	5
Emerald shiner	0.86	0.29	6
Golden shiner	0.34	.	1
Logperch	0.32	.	1
Round goby	0.07	.	1
Pumpkinseed	0.32	0.15	3
Rainbow smelt	0.30	0.17	24
Rock bass	0.26	.	1
Slimy sculpin	0.18	0.03	2
Spottail shiner	0.44	0.18	12
Trout-perch	0.29	0.17	16
Yellow perch	0.30	0.12	20

[1]For species captured in more than one lake, means of lake-specific least-squared means were calculated (standardized mass of 8g). For species captured in only one lake, least-squared means (standardized mass of 8g) are presented for that lake.

Table 4.4. Summary statistics of intraspecific linear regressions between Hg concentration and. $\log_e$ wet mass[1]

Species	Slope	Intercept	P-value
Cisco	0.013	0.234	0.2301
Emerald shiner	0.127	0.526	0.0079*
Rainbow smelt	0.087	0.102	0.0001*
Spottail shiner	0.100	0.224	0.0009*
Trout-perch	0.102	0.088	0.0001*
Yellow perch	0.085	0.115	0.0001*

[1]Among-lake variation in Hg concentrations was removed prior to analysis (see methods).

*Regressions are significant at α=0.05.

Table 4.5. Summary of Pearson product-moment correlations between intraspecific $\log_e$ Hg concentrations and biotic variables

Species	Variable[1]	Correlation coefficient	P-value
Cisco	trophic position	-0.80	0.101
Emerald shiner	$\delta^{13}C$	-0.73	0.101
Rainbow smelt	$\log_e$ growth rate	-0.52	0.010*
	$\delta^{13}C$	-0.45	0.030*
Spottail shiner	$\delta^{13}C$	-0.42	0.176
Trout-perch	$\log_e$ growth rate	-0.45	0.078
Yellow perch	$\log_e$ growth rate	-0.51	0.022*
	$\delta^{13}C$	-0.43	0.059

[1]The variables presented are those that were most closely related to intraspecific $\log_e$ Hg concentrations. If nothing was significant, the variable with the lowest P-value is shown. All correlations are based on least-squared means calculated at a standardized mass of 8g.

*Relationships are significant at α=0.05.

Table 4.6. Summary of Pearson product-moment correlations between intraspecific $\log_e$ Hg concentrations and abiotic variables

Species	Variable[1]	Correlation coefficient	P-value
Cisco	maximum depth	0.65	0.166
Emerald shiner	conductivity	-0.92	0.027*
	$\log_e$ area	-0.96	0.009*
Rainbow smelt	conductivity	-0.65	0.003*
Spottail shiner	conductivity	-0.69	0.056
Trout-perch	conductivity	-0.67	0.017*
	pH	-0.65	0.031*
Yellow perch	conductivity	-0.57	0.021*

[1]The variables presented are those that were most closely related to intraspecific $\log_e$ Hg concentrations. If nothing was significant, the variable with the lowest P-value is shown. All correlations are based on least-squared means calculated at a standardized mass of 8g.

*Relationships are significant at α=0.05.

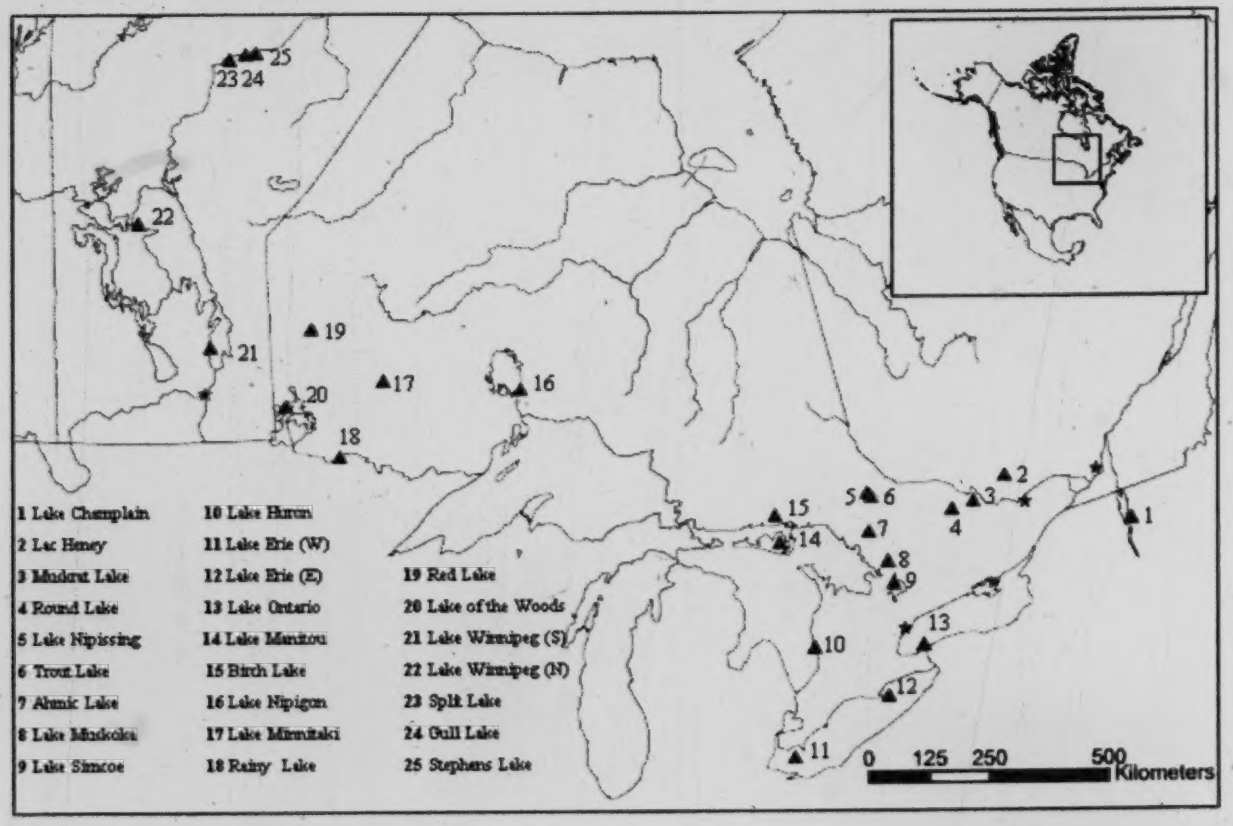

Figure 4.1. Map of the study lakes.

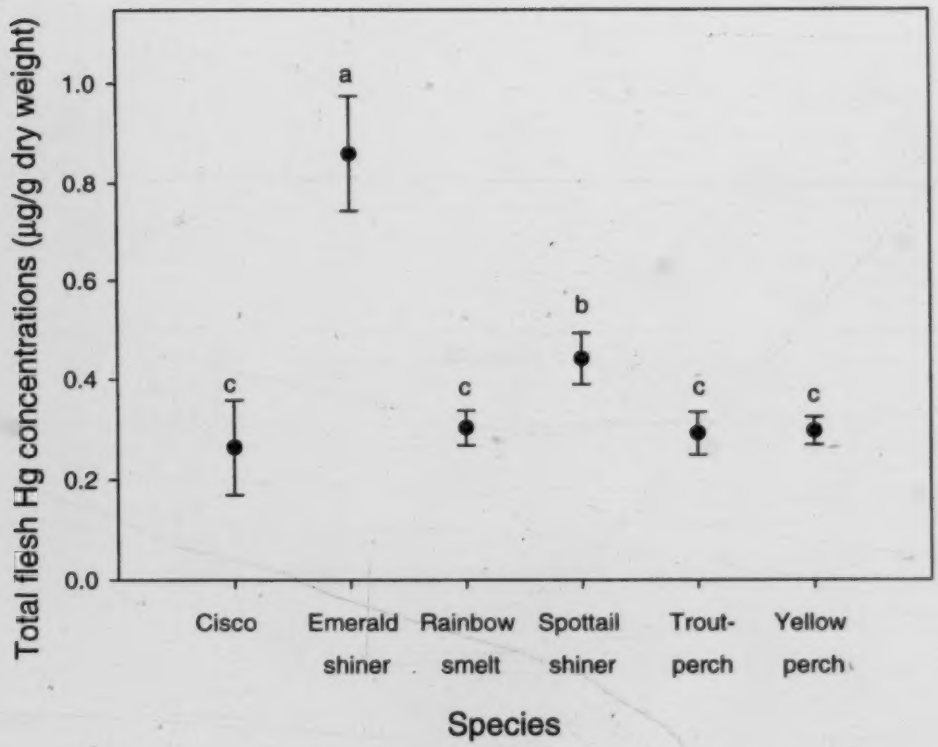

Figure 4.2. Least-squared mean (standardized mass of 8g) total Hg concentrations ± SE. Emerald shiner had significantly higher Hg concentration than all other species, and spottail shiner had a significantly higher Hg concentration than cisco, rainbow smelt, trout-perch, and yellow perch (Tukey's test, $P<0.05$). Letters indicate significant pair-wise differences.

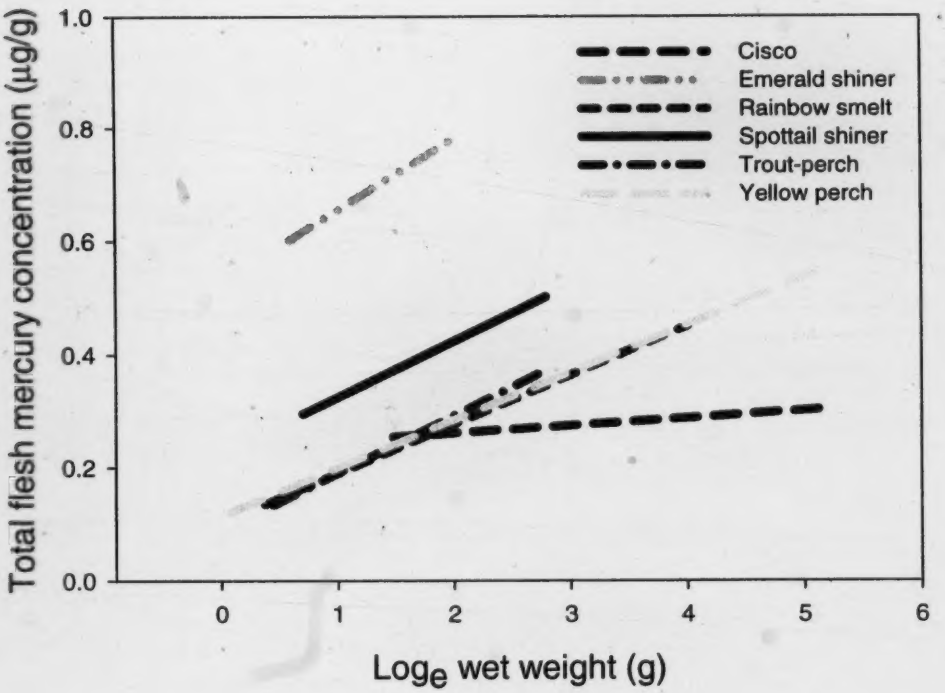

Figure 4.3. Species-specific linear regressions of total [Hg] vs. $\log_e$ wet mass. Data used to obtain the relationships were corrected for among-lake differences but individual data points are not shown for clarity (N=753). Slopes were significantly different among species (ANCOVA, F=3.78, P=0.0022, df=5,741). It does not appear, however, that changing the size of standardization would alter the inter-species comparison.

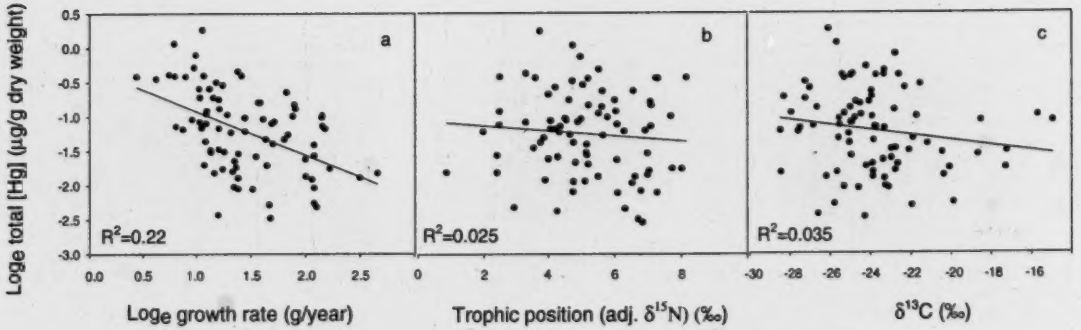

Figure 4.4 a,b,c. Interspecific (all species pooled) linear regressions of total Hg concentrations vs. growth rate, $\delta^{15}N$-determined trophic position, and $\delta^{13}C$, respectively. When all three variables are entered in a multiple regression to explain variation in Hg concentrations, growth rate explains the vast majority of the variation (R^2=0.22 (growth rate), 0.025 (trophic position), and 0.035 ($\delta^{13}C$). Values plotted are least-squared means calculated for each species in each lake at a standardized mass of 8g.

Chapter 5: General Conclusions

Consistent with previous stable isotope studies, I suggest that rainbow smelt are trophically elevated relative to the majority of other forage fish species and are most likely to overlap in habitat with pelagic-dwelling species such as cisco and emerald shiner (Evans and Loftus 1987, Franzin et al. 1994, Overman and Parrish 2001, Swanson et al. 2003). Based on baseline $\delta^{15}N$ and $\delta^{13}C$ analyses, I also suggest that Lake Winnipeg (south basin), Lake Erie (east basin), and Lake Ontario receive high amounts of anthropogenically-derived nitrogen and that dissolved inorganic carbon sources vary along gradients of lake size. It appears that larger lakes receive isotopically heavy inorganic carbon from sources such as weathered rock and atmospheric CO_2 and that smaller lakes receive isotopically light inorganic carbon from the respiration and re-mineralization of internal and terrestrial organic matter.

Data on forage fish growth patterns and growth rates are sparse because ageing is often difficult in small fish species and because forage fish often have no direct commercial value. For species where comparisons were possible, the isometric growth patterns observed in this study were reasonably consistent with previously published results (Beckman 1942, Baten and Tack 1952, Swingle 1965, Carlander 1969). Inter-species comparisons of growth rate revealed that cisco and yellow perch had the highest absolute growth rates, trout-perch and rainbow smelt were intermediate, and the two shiner species were lowest. These differences corresponded to differences in theoretical maximum size (Froese and Pauly 2003). Within species, rainbow smelt and trout-perch growth rates were positively related to conductivity, and spottail shiner and yellow perch growth rates were negatively related to latitude. These results may reflect effects of temperature, length of growing season, and food availability on growth.

In agreement with Swanson et al. (2003), the results of this study indicate that $\delta^{15}N$-determined trophic position is not a good predictor of forage fish [Hg]. Forage fish [Hg] was most closely related to growth rate and lake conductivity. Similar results have been presented for yellow perch (Greenfield et al. 2001), but this is the first study that has related Hg concentration, trophic position (determined by stable isotopes), growth rate, and lake characteristics in multiple forage fish species.

The weak relationship observed between trophic position and forage fish [Hg] is most likely due to the limited longevity of forage fish and the relatively small differences in trophic position among species. If a predator species had been included in this study a biomagnification effect would almost certainly have been detected. This raises questions of scale. How much variation in trophic position is needed to see a significant effect on [Hg]? Is there a threshold lifespan where trophic position is a better predictor of [Hg] than growth rate or water chemistry? Both of these questions require further research.

The results of this study have implications for predicting the impacts of rainbow smelt invasion on predator fish [Hg]. It appears that pre-to post-invasion diet switches to rainbow smelt should only result in predator [Hg] increases if predators switch from a cisco-dominated diet to a rainbow smelt-dominated diet. The most likely predator to experience this is lake trout, a long-lived pelagic predator. Thus, it is not surprising that previous post-invasion [Hg] increases have most often been reported in lake trout (Akielaszek and Haines 1981, MacCrimmon et al. 1983, Vander Zanden and Rasmussen 1996). Predator [Hg] could also increase pre- to post-smelt invasion in systems where rainbow smelt grow more slowly than other forage fish species. In this study, rainbow smelt growth was positively correlated to conductivity. Other authors have found that smelt grow more slowly in shallow systems that lack thermal refugia (e.g., south basin Lake Winnipeg) (Franzin et al. 1994). Predator [Hg] increases following smelt invasion may therefore be more likely in shallow, warm systems, or systems with low conductivity. Future research should include a more extensive investigation of factors that determine relative growth rates in rainbow smelt-invaded forage fish communities, such as food availability, food quality, and density of conspecifics and competitors.

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Appendix A: Summary of mercury concentrations

Table A.1 Mean total Hg concentrations by lake and species

Species	Lake	Total Hg concentration (µg/g)	Standard deviation
Alewife	Huron (south)	0.24	0.04
Alewife	Ontario (west)	0.17	0.04
Bluegill	Muskrat	0.24	0.02
Cisco	Champlain	0.18	0.02
Cisco	Manitou	0.24	0.04
Cisco	Minnitaki	0.40	0.10
Cisco	Nipigon	0.56	0.19
Cisco	Trout	0.30	0.07
Cisco	Winnipeg (north)	0.10	0.03
Cisco	Winnipeg (south)	0.12	0.03
Common shiner	Heney	0.59	0.11
Common shiner	Manitou	0.26	0.13
Common shiner	Muskoka	0.51	0.12
Common shiner	Muskrat	0.45	0.12
Common shiner	Round	0.37	0.10
Emerald shiner	Gull	0.68	0.16
Emerald shiner	Minnitaki	1.22	0.19
Emerald shiner	Nipissing	0.55	0.15
Emerald shiner	Red	0.82	0.25
Emerald shiner	Winnipeg (north)	0.51	0.09
Emerald shiner	Winnipeg (south)	0.56	0.12
Round goby	Ontario (west)	0.08	0.03
Golden shiner	Ahmic	0.34	0.05
Logperch	Nipissing	0.32	0.08
Pumpkinseed	Ahmic	0.34	0.05
Pumpkinseed	Muskoka	0.49	0.07
Pumpkinseed	Simcoe	0.16	0.04
Rock bass	Manitou	0.25	0.05
Rainbow smelt	Ahmic	0.53	0.12
Rainbow smelt	Birch	0.49	0.16
Rainbow smelt	Champlain	0.41	0.22
Rainbow smelt	Erie (east)	0.18	0.10
Rainbow smelt	Gull	0.23	0.10
Rainbow smelt	Heney	0.23	0.15

Rainbow smelt	Huron (south)	0.15	0.07
Rainbow smelt	Manitou	0.11	0.03
Rainbow smelt	Minnitaki	0.28	0.12
Rainbow smelt	Muskoka	0.70	0.34
Rainbow smelt	Muskrat	0.40	0.10
Rainbow smelt	Nipigon	0.27	0.17
Rainbow smelt	Nipissing	0.15	0.02
Rainbow smelt	Ontario (west)	0.25	0.09
Rainbow smelt	Rainy	0.37	0.14
Rainbow smelt	Red	0.20	0.08
Rainbow smelt	Round	0.50	0.20
Rainbow smelt	Simcoe	0.17	0.07
Rainbow smelt	Split	0.16	0.14
Rainbow smelt	Stephens	0.22	0.09
Rainbow smelt	Trout	0.46	0.26
Rainbow smelt	Winnipeg (north)	0.13	0.03
Rainbow smelt	Winnipeg (south)	0.08	0.01
Rainbow smelt	Woods	0.29	0.05
Slimy sculpin	Ontario (west)	0.21	0.05
Slimy sculpin	Simcoe	0.14	0.05
Spottail shiner	Erie (east)	0.24	0.07
Spottail shiner	Gull	0.50	0.22
Spottail shiner	Manitou	0.27	0.07
Spottail shiner	Nipigon	0.76	0.11
Spottail shiner	Nipissing	0.41	0.07
Spottail shiner	Ontario (west)	0.31	0.14
Spottail shiner	Rainy	0.45	0.11
Spottail shiner	Red	0.63	0.15
Spottail shiner	Round	0.51	0.16
Spottail shiner	Winnipeg (north)	0.29	0.08
Spottail shiner	Winnipeg (south)	0.35	0.19
Spottail shiner	Woods	0.29	0.06
Trout-perch	Birch	0.56	0.33
Trout-perch	Erie (east)	0.16	0.13
Trout-perch	Erie (west)	0.31	0.12
Trout-perch	Gull	0.22	0.05
Trout-perch	Heney	0.18	0.08
Trout-perch	Manitou	0.18	0.06
Trout-perch	Minnitaki	0.53	0.17
Trout-perch	Muskrat	0.28	0.12
Trout-perch	Nipigon	0.42	0.20
Trout-perch	Nipissing	0.31	0.18

Trout-perch	Rainy	0.15	0.04
Trout-perch	Red	0.38	0.06
Trout-perch	Round	0.45	0.09
Trout-perch	Winnipeg (north)	0.14	0.13
Trout-perch	Winnipeg (south)	0.30	0.10
Trout-perch	Woods	0.13	0.00
Yellow perch	Ahmic	0.35	0.05
Yellow perch	Birch	0.62	0.25
Yellow perch	Champlain	0.90	0.30
Yellow perch	Erie (east)	0.36	0.23
Yellow perch	Erie (west)	0.22	0.03
Yellow perch	Heney	0.10	0.03
Yellow perch	Huron (south)	0.28	0.04
Yellow perch	Manitou	0.16	0.10
Yellow perch	Minnitaki	0.40	0.15
Yellow perch	Muskoka	0.48	0.26
Yellow perch	Muskrat	0.29	0.16
Yellow perch	Nipigon	0.36	0.16
Yellow perch	Nipissing	0.27	0.14
Yellow perch	Rainy	0.40	0.09
Yellow perch	Red	0.34	0.09
Yellow perch	Round	0.37	0.08
Yellow perch	Simcoe	0.15	0.09
Yellow perch	Trout	0.41	0.20
Yellow perch	Winnipeg (north)	0.15	0.04
Yellow perch	Winnipeg (south)	0.31	0.36
Yellow perch	Woods	0.41	0.14